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RESEARCH—DOORWAY TO THE FUTURE

SUMMARY OF REMARKS BY EARL L. BUTZ

Dean of Agriculture, Purdue University, Lafayette, Indiana, before the 49th Annual Meeting of the American Society of Animal Production, Chicago, Illinois, November 30, 1957

LIVESTOCK producers now use large amounts of science and technology. Profits are increasingly dependent upon one's ability to keep abreast with research developments in breeding, in nutrition, in disease control, and in a dozen related fields.

The remarkable thing about science in the livestock pen is that it has now set the stage for revolutionary changes in production and marketing.

Research has given us animals tailored to market demand, and formula feeds that can be efficiently converted into meat. Research has also given us better methods of disease control, new ideas and equipment for labor saving, and techniques for stepping up yields per breeding unit.

The advances you scientists are producing on the livestock front are but a part of the great American Dream.

A Great Future Lies Ahead

This is the age of science and technology, based on research. The frontiers of the mind have replaced the frontiers of geography. A thrilling experience awaits every citizen in this great land who has the capacity and the imagination to "dream constructively".

The scientific and technological advances we will experience in the next decade will be unparalleled in American agriculture and industry.

The geographic frontier in America is gone. No longer can a young man "Go West" and stake out his claim. But the scientific frontier in America is barely scratched. And the scientific frontier has no effective limit. It is limited only by the mind and the imagination of man. Its horizons are vertical, not horizontal.

Organized and imaginative research is the vehicle which will push the scientific frontier beyond limits we scarcely dare dream of today. It follows logically, therefore, that if we can keep our economy free and preserve an environment in which individual producers and scientists are free to dream a little about new techniques and new ideas, and to enjoy the fruits of their dreams, we shall experience phenomenal progress in the next generation.

It is to this noble purpose that you who sit before me dedicate your energies.

This is a thrilling time to be permitted to live in this great land we call America. Yours is a wonderful opportunity to occupy a front-row seat for the scientific advances occurring in our Land Grant Colleges and Universities, in the U.S.D.A., in our research foundations and laboratories, and in our agricultural organizations.

Our livestock enterprises afford an excellent illustration of an industry in the very middle of a far-reaching scientific and technological revolution.

Changes which have occurred in production and marketing during the past ten years have been phenomenal. Still greater changes lie ahead.

American agriculture is now feeding our growing population on science and technology. Today the average farm worker in the United States produces enough food and fiber for himself and 19 others. A generation ago, in 1930, he produced enough for himself and only 9 others. A century previously, in 1830, one farm worker produced enough for himself and 3 others.

Technological changes have resulted in startling increases in output per acre, per animal, and in total farm output. The physical volume of output of U. S. farms in 1956 was about 50% above a quarter of a century ago. The larger output was produced with about a third fewer farm workers. This means that the output per worker in American agriculture has more than doubled in the last quarter century. This is a record of increased efficiency that can be matched in very few places in the American economy. This has resulted in a higher level of living for farm families, and cheaper food for urban families.

Some of the research developments that have made this expansion possible are truly spectacular. You members of the American Society of Animal Production are thoroughly familiar with the achievements that have been made in plant and animal nutrition, in selection and breeding of strains especially suited to particular environmental factors and resistant or immune to disease, in ever-lowering feed conversion ratios, and the far-reaching research that has been done in processing and marketing, and in market development.

It would be possible to fill these pages with specific illustrations of research that has returned its cost an hundredfold. But our interest is in the future. Let's dream a little about that.

Am I Changing with the Times?

The most constant thing about our society is change. Ours is a dynamic economy. This is especially true of American agriculture. It must also be true of our trade and scientific association programs, our college research programs, and our federal agricultural programs.

We must always ask ourselves, "Am I changing and is my program changing so as to make a maximum contribution to the changing environ-

ment in which I and my organization must operate? Or do I cling to an obsolete set of standards, wornout methods of doing business, an antiquated model of operation?"

These are days when research and industry leaders need greater vision than ever before. They need to spend more time in constructive dreaming than many of us have the inclination or the time to do.

Never before in history has the future been so close to us as now. Research and education are shortening the time span of progress. We shall occupy ringside seats in scientific and technological developments during the next ten years equivalent to changes which our fathers took a generation to accomplish.

This prospect was pretty well summed up a while back by a Washington taxi driver. As he drove down Pennsylvania Avenue his passenger read aloud the inscription on the face of the National Archives Building: "What is past is prologue." He inquired of the cab driver what that meant. Back came the reply: "That means, you ain't seen nothing yet."

So it will be with your work and mine in the next decade.

The forces of innovation now in motion promise accelerated increases in crop and livestock yields, and tremendous developments in distribution and marketing. As diseases and pests come under more complete control, as applied genetics increases production per acre and per animal, as future mechanical devices are put into use, as new market efficiencies are introduced, the output per man in agriculture will continue to expand rapidly.

All of these developments point to the need for closer cooperation among farmers, agricultural businesses serving farmers, and educational and research people. As greater specialization develops, need grows for some planning to avoid unbalance and potential surpluses. Wise planning will emphasize choices in the direction of quality improvements that will produce a more diversified array of farm products.

Technological changes of the kind and rapidity we envision here can only mean an accelerated rate of social and economic adjustment in our rural communities. This will be neither easy nor painless. The best leadership among you will be challenged to guide these adjustments along constructive and beneficial pathways.

Some who worship at the shrine of "staus quo" will seek to thwart adjustments which are inevitable in a dynamic and growing society. They will impede progress, and sometimes make the adjustment more painful when it does become inevitable.

Others, with limited vision, will follow the adjustments blindly. They will neither help nor hinder.

I am confident that the leaders of our great livestock industry will have the breadth of imagination and the clarity of vision to direct our modern agricultural revolution down the pathway of a progressive, prosperous, and free agriculture in a growing, dynamic, and free America.

There's a Big Job Ahead of Us

In the short span of ten years, our population has increased by about 25 million to the current figure of 171 million persons. By 1975 it is estimated that the number will be over 220 million. And equally significant, it is estimated that by 1975 the national economy will have about doubled in size, and that per capita real income will be around 50 to 60% higher than at present. Such a rise will bring about a substantial increase in the demand for all goods and services, including foods. That branch of the food industry that keeps up with the march of progress through research will capture more than its pro rata share of this growing market.

The rapidly growing U. S. population, combined with a significant uptrend in real purchasing power of our people, foretells a marked expansion in the market for meat and meat products in the years ahead.

One per capita real income is now about one-tenth above ten years ago and two-thirds above twenty years ago. It is expected to increase about one-fifth in the next decade and by 1975 will be about 50% higher than now.

Although it is true we do not increase our food consumption proportionately as income increases, we do "eat higher on the hog". We improve the quality of our diet. We do this by shifting to meat, milk, eggs, and other more expensive protective foods.

It has been estimated that by 1975 we will need to produce, process, and market 1/3 more beef than now, 2/5 more pork, 1/6 more lamb, 2/5 more broilers and chickens, 1/3 more turkeys, 1/2 more eggs, and 1/4 more milk. All livestock and livestock products will need to increase by more than 1/3.

All this adds up to growing emphasis on what has come to be known as "animal agriculture". To the extent that we succeeed in merchandising our farm production into human consumption in the form of meat, milk, and eggs, we will have moved a long way towards solution of the problem of chronic surpluses of feed grains which plague contemporary agriculture and confuse agricultural policy.

On the Threshold of Discovery

We appear to be on the verge of a period of unusual development in livestock production, as new discoveries in breeding and nutrition make possible increases in efficiency not thought possible before.

Radioisotopes are playing an important role in fundamental research in animal metabolism, which will contribute to lower cost production through improved feeds and better management practices.

In the period ahead it will be possible to produce a great many quality changes that will reduce labor and expense, or fill particular needs of consumers more adequately.

About 70% of all crop acreage now is planted to varieties not even in existence on a commercial scale 20 years earlier. We may expect this process to accelerate as atomic energy radiation is used to speed up the rate of occurrence of mutations.

Today we cultivate only a portion—a little more than 1%— of the 15,000 plant species that are native to this country. In the world at large there are some 250,000 species. Most of the cultivated species were selected in the dim time before recorded history. But once in a while we find something in this vast biological storehouse that can be put to new use. Sometimes, like the soybean, it is known elsewhere. Sometimes it is little known —an almost but not quite, profitable crop that a twist of fate or shift in genetic makeup will push over the boundary into the profitable area. It is the informed mind, the not being blind to seemingly unimportant things, that pays off on the long chance in this area.

With livestock it is possible to improve and modify such qualities as tenderness, flavor, wool, feather color, and many others to the advantage of the farmer and the consumer. More of this will take place. The commercial broiler is a prototype of the kind of process that may occur. New synthetic amino acids, hormones, antibiotics and other growth stimulants or aids are coming along.

The research people among the members of the American Society of Animal Production will have a lot to do with shaping the future of agriculture. Research people everywhere recognize that the job ahead will require the hardest kind of work. The easy questions have already been answered. Many of yesterday's research problems have been solved by relatively simple, straightforward, and fairly obvious methods. Those that face us today are highly complex. We must continually search for new tools with sharper cutting edges, more potent materials, and new ways of using them if we are to get the job done.

The point should be made that potential markets influence almost every phase of agricultural research. The demand for the enormous variety of commodities in stable supply guides research in production as well as in processing and distribution. It helps to shape plans not only for investigations that pay off with techniques for immediate use, but also for the long-time fundamental studies that blaze the trail for further research.

No Moratorium on Research

We must all combat the philosophy that sometimes rears it ugly head and asserts that because of current agricultural surpluses we should "declare a moratorium on research and education".

This is a false and dangerous doctrine. It was preached in 1920 when post World War I surpluses developed. Think where we woud be today if that philosophy had prevailed 25 years ago. It was preached again in 1940, just before World War II, when surpluses again plagued us. Think

for a moment where we would be today if we had listened to that just 15 years ago. The best way today to stop the steady march toward fulfillment of the American Dream would be to pull the rein on research and education. This we must never do.

We talk a lot in America about horsepower. However, our greatest national resource today is brainpower. This complex scientific and social environment in which we live demands increasingly competent men and women to manage it effectively. We must develop the brainpower of young America in such a way that the generation ahead can enjoy fully the technological and social developments which await us. Not to train to its highest capacity the brainpower of our young men and women in America is just as wasteful of one of our great natural resources as not to exploit a new pit of iron ore, a new bed of uranium, or our fertile fields.

We must be careful in the current wave of hysteria over Sputniks, Mutniks, and the like that we don't become lopsided in our training programs in high schools and colleges. To be sure, we need competent chemists, physicists, and engineers. But we don't need them exclusively. We need other scientists also. We must be careful that we don't unbalance our training to the same extent that Russia has done. They have the physicists and chemists, but they are woefully short of competent personnel in other important areas. Russia has trained her young people for the science of destruction. We have trained ours for the art of living. We must maintain balance in our training program if we are to continue in the years ahead toward fulfillment of the American Dream.

Free Men with Vision Will Do the Job Best

In recent years a great many sectors of the American agricultural industry have fallen under governmental controls and restrictions. These controls have followed in the wake of price supports at uneconomic levels.

The livestock industry, in the main, has steered clear of any such pitfall as this. Your leaders have recognized that when a commodity gets itself into the fix of producing for the government rather than producing for a growing market, it almost inevitably finds a ceiling placed on opportunity.

Government production and marketing controls are essentially backward looking—not forward looking. Under these circumstances, producers with above-average managerial capacity and ambition are severely limited in what they can do. They suffer, consumers suffer, and all America suffers.

The future of the livestock industry is closely tied in with increased efficiency of production, processing, and distribution, through which you can reach an ever-growing circle of consumers in the market place, here and abroad. Private enterprise must exercise the initiative in getting this job done.

You must strive ever to preserve a proper relationship between your industry and government. You must always keep private enterprise the

senior partner, and government the *junior* partner. It would be easy to reverse this relationship. There are many who would change it. You must be ever vigilant that your industry assumes the responsibilities put upon it by our private enterprise system. Otherwise government *will* become the senior partner. This is inherent in the very nature of government.

You must constantly remind yourself that the advances of science can be applied most effectively by individual managers in a free industry, unhampered by excessive governmental regulation and restriction.

You must keep before you always the concept that livestock, like other farm products, is produced to be consumed in useful outlets, and not diverted into purposeless storage or uneconomic uses through politically inspired governmental price support programs.

We must all work together to preserve an atmosphere in which freedom of choice remains one of the basic pillars of our economic system. Under such a system, individual producers and individual processors can grow and prosper as far as their ambition and their ability will take them. The right to succeed will be open to everyone. Conversely, the right to fail will also be present.

In our free society, the right to succeed carries with it the right to fail. If, through legislative action of one kind or another, we remove the right to fail, we ultimately will also remove the right to succeed beyond mediocrity. Men of vision and ambition do not want that. They know their future is most secure in an environment which guarantees freedom to choose, freedom to experiment, freedom to become more efficient, freedom to seek and develop new markets, freedom to dream, and freedom to enjoy economic rewards if their dream is successful.

One of the great challenges facing all of us is to see that our economy is not dominated by government—that government helps rather than displaces private enterprise. We can do this only if we are willing to throw our influence on the side of keeping government the servant of all of us —not our master.

UTILIZATION AND/OR SYNTHESIS OF VALERIC ACID DURING THE DIGESTION OF GLUCOSE, STARCH AND CELLULOSE BY RUMEN MICRO-ORGANISMS *IN VITRO*[1, 2]

JACK H. CLINE, T. V. HERSHBERGER[3] AND ORVILLE G. BENTLEY

Ohio Agricultural Experiment Station, Wooster

THE role of the rumen microflora in the formation of volatile fatty acids (VFA) from feedstuffs, especially carbohydrates and proteins, during ruminal digestion is well established. Certain fatty acids, however, appear to be specific nutritional requirements of the rumen microflora itself. Bentley *et al.* (1955a) reported that 5- and 6-carbon volatile fatty acid and iso-butyric stimulated the growth of rumen micro-organisms *in vitro* as determined by increased cellulose digestion and non-protein nitrogen assimilation. Bryant and Doetsch (1955) found that a combination of straight and branched chain 5- and 6-carbon fatty acids were required for the growth of cellulolytic rumen organisms, *Bacteroides succinogenes.* These observations would link fatty acids produced in the rumen to the nutrition of the microflora *per se.* It became of interest to determine the effect of various nutrients needed for growing rumen micro-organisms *in vitro* in a purified medium on the synthesis and requirement of VFA. Studies involving the effect of changing the carbohydrate substrate (starch, cellulose, or glucose) and the levels of urea nitrogen in the medium on valeric acid synthesis and/or utilization by the rumen micro-organisms are reported herein.

Experimental

The *in vitro* rumen fermentation system and medium previously described by Bentley *et al.* (1955a) was used. In preparing the inoculum, the sediment from rumen liquor was collected on a celluloid liner placed in the cylinder of a Sharples supercentrifuge. The sediment on the bottom inch of the celluloid liner, composed primarily of plant debris, protozoa and some bacteria, was discarded. The remainder of the sediment was resuspended in phosphate buffer (pH 7.0) and the equivalent of 40 ml. of strained rumen juice used to inoculate each flask (10 ml. of the suspension). When this method of preparing the inoculum was used, little cellulose digestion occurred in the control flasks (the flasks without added valeric

[1] From a dissertation presented by Jack H. Cline to the Graduate School of The Ohio State University in partial fulfillment of the Ph.D. degree, 1956.

[2] Published with the approval of the Associate Director, Ohio Agricultural Experiment Station as Journal article No. 69–56. Supported in part by Grants-in-Aids from Swift and Company, Chicago, Illinois, and the Commercial Solvents Corporation, Terre Haute, Indiana.

[3] Present address: Department of Animal Nutrition, Pennsylvania State University, University Park, Pa.

acid) while as much as 1 gm. of cellulose was digested in the flasks containing the supplement.

Cellulose analyses were performed using the volumetric method of Hershberger *et al.* (1955). Protein synthesis was determined by analyzing for nitrogen the precipitate formed by adding 5 ml. of 50% trichloroacetic acid to 20 ml. of the fermentation mixture (final acid concentration, 10%). The mixture was allowed to stand 18 to 24 hr., the precipitate separated by centrifuging for 15 min. at about 1900 r.p.m. in a No. 2 International centrifuge, the supernatant siphoned off and the precipitate washed with

TABLE 1. THE EFFECT OF THE LENGTH OF FERMENTATION ON THE *IN VITRO* VALERIC ACID CONTENT USING CELLULOSE

Length of fermentation, hours	Valeric acid, mg./100 ml.	
	Experiment 1[a]	Experiment 2[b]
0	17.6	5.6
½	....	4.6
1	....	5.2
2	....	5.2
5	....	4.6
12	10.4	4.6
20	....	2.8
21	5.7	...
30	4.2	2.4

[a] 15.6 mg. added valeric acid per 100 ml.
[b] 5.6 mg. added valeric acid per 100 ml.

10 ml. of 10% trichloroacetic acid. After centrifuging as before, the precipitate was transferred to the Kjeldahl flasks for nitrogen analysis. Nitrogen determined in this manner will be referred to as T.C.A.-N. and represents the increase in trichloroacetic acid insoluble nitrogen-containing material in the flasks (bacterial protein) during the fermentation. Valeric and caproic acid analyses were performed chromatographically using a modification of Harper's (1953) method as described by Bentley *et al.* (1955a), which will not separate valeric acid from its branched-chain isomers.

Results

In preliminary experiments, the level of valeric acid in the *in vitro* fermentation medium containing 1% cellulose was determined and the results are given in table 1. The valeric acid content of the medium did not change appreciably until about the 12th hour of the fermentation at which time it began to decrease. Although the initial level of added valeric acid was different for these two experiments, the same trend of decreasing valeric acid levels with fermentation time was observed between the 12th hour and 30th hour.

TABLE 2. THE VALERIC AND CAPROIC ACID CONTENT OF RUMEN JUICE SUPERNATANT AS AFFECTED BY TIME AFTER FEEDING, MG. PER 100 ML.[a]

Acid	Hours after feeding			
	0	3	6	9
Caproic	4	8	2	4
Valeric	21	35	23	22

[a] Sharples supercentrifuge supernatant of rumen fluid.

In previous experiments where time-activity curves for cellulose digestion had been determined, it was found that the most rapid cellulose digestion occurred during this same time interval, i.e., between the 10th and 30th hour. That valeric acid levels in rumen fluid could vary as the result of dietary changes is indicated by the work of Annison, (1954) and Gray *et al.* (1952). Time after feeding can also influence the levels of valeric and caproic acid in the rumen juice as suggested by the analyses for these acids reported in table 2.

The use of starch and cellulose as the carbohydrate substrate for rumen micro-organisms alters the nature of fermentation as indicated by a more rapid drop in pH, more total fatty acids and, in some cases, more rapid cellulose digestion (Arias *et al.*, 1951; Belasco, 1956; Pounden and Hibbs, 1948, 1949; Hunt *et al.*, 1954; Conrad *et al.*, 1956). It was of interest therefore to determine the valeric acid content of the *in vitro* fermentation flasks when starch was a part of the substrate. The results shown in table 3, indicate that the total valeric acid content of the medium was about the same at the end of the experiment as at the beginning in this type of fermentation in contrast to one wherein cellulose was the major substrate as shown in table 1. Apparently, valeric acid was formed by the starch-digesting organisms as 8.2 mg. was found at the end of 30 hr. and nearly all the added valeric acid was recovered from a similar flask in the same experiment.

To verify further these preliminary observations two experiments were run about a year later. The treatments were duplicated within each experi-

TABLE 3. THE EFFECT OF VALERIC ACID ON PROTEIN SYNTHESIS BY STARCH DIGESTING MICRO-ORGANISMS

Additions to basal medium[a]	Increase in T.C.A.-N, mg./100 ml.	Valeric acid mg./100 ml.
1.0 gm. starch	17.0	8.2
1.0 gm. starch + 5.6 mg. valeric acid	17.2	13.2

[a] Basal medium with cellulose omitted.

ment. The average values for each treatment for the T.C.A.-N formation, cellulose digested and the level of valeric acid in the medium from two experiments are given in table 4.

Again it was observed that after 30 hr. with the cellulose substrate, no valeric acid was present in the medium (no. 1) but if 0.5 gm. starch were added with the cellulose or 1.0 gm. starch substituted for cellulose, considerable valeric acid was found in the medium, nos. 3 and 5. The addition of 29 mg. of valeric acid with cellulose at the beginning of the experiment increased the valeric acid at the end of the experiment but only

TABLE 4. STARCH AND VALERIC ACID ADDITIONS TO THE MEDIUM AND THE AMOUNT OF CELLULOSE DIGESTION, THE VALERIC ACID CONTENT OF THE MEDIUM, AND BACTERIAL PROTEIN FORMATION BY RUMEN MICRO-ORGANISMS *IN VITRO* [a]

Treatment no.	Additions to 100 ml. basal, medium	9/17/57			9/24/57		
		T.C.A.-N, mg./ 100 ml.	Cellulose digested, gm.	Valeric acid, mg./ 100 ml.	T.C.A.-N, mg./ 100 ml.	Cellulose digested, gm.	Valeric acid, mg./ 100 ml.
1	None [a]	12.6	0.40	0	7.6	0.18	0
2	29.98 mg. valeric acid (V.A.)	18.5	1.15	20.7	19.3	1.07	19.3
3	0.5 gm. starch	21.1	0.91	5.5	19.8	0.64	1.7
4	V.A. plus 0.5 gm. starch	23.8	0.89	29.2	22.9	0.97	30.4
5	1.0 gm. starch	16.3	0	9.6	12.6	0	6.9
6	1.0 gm. starch plus V.A.	17.0	0	41.2	12.9	0	40.0

[a] 2% cellulose was added to the medium (100 ml.) in treatments 1 through 4. No cellulose was added to the medium for treatments 5 and 6.

67% was "recovered", No. 2. However, in the presence of 0.5 gm. starch (no. 4) or 1.0 gm. starch (no. 6), 100% and 135%, respectively, of the added acid was "recovered". An analysis of variance was run on these data (table 4) and the least significant difference (L.S.D.) between treatments calculated. It was found that a significant difference (5% level) existed between experiments but even with this variation a highly significant difference (1% level) existed between treatments for cellulose digested, and valeric acid and T.C.A.-N content. The calculated L.S.D. values indicate that (a) there was a highly significant increase in microbial protein for the valeric acid, 0.5 gm. starch or 1 gm. starch treatments and the difference between treatments 2 and 3, table 4, was not significant; (b) a significant increase in cellulose digested occurred on addition of valeric acid, but no significant difference due to addition of 0.5 gm. starch; (c) a highly significant difference in valeric acid content occurred between treatments 1 and 5, 2 and 4, 2 and 6, and 4 and 6 while the difference between 1 and 3 was not significant.

The results of these experiments confirm the earlier observations, namely, that during starch digestion by the rumen microflora (amylolytic organisms), the amount of valeric acid formed increased; whereas, when

cellulose was the only carbohydrate, either the rate of utilization exceeded the rate of synthesis or the acid was not produced at all.

Further indication of the carbohydrate-valeric acid synthesis relationship is indicated by the results from a single experiment wherein glucose was used as the "more readily available" carbohydrate instead of starch, table 5. Again a low valeric acid level was observed when cellulose was the major carbohydrate while considerable valeric acid, 7.4 mg. per 100 ml. was found if 1 gm. of glucose replaced the cellulose. This suggests that the metabolic pathways for glucose (an intermediate in starch hydrolysis) and starch are similar with respect to valeric acid utilization and/or formation.

TABLE 5. THE SYNTHESIS OF VALERIC ACID AND BACTERIAL PROTEIN BY RUMEN OFGANISMS *IN VITRO* USING GLUCOSE SUBSTRATE

Carbohydrate substrate	T.C.A.-N, mg./100 ml.	Valeric acid mg./100 ml.
Cellulose, 1 gm.	5.4	0.4
Cellulose + 15.6 mg. valeric acid per flask	8.9	13.6
Glucose, 1 gm.	5.7	7.4
Glucose + 15.6 mg. valeric acid	10.9	22.2

Hershberger (1955a) found that cellulose digestion and T.C.A.-N formation by the rumen micro-organisms grown on a medium comparable to that used herein were related to the urea nitrogen level in the flasks when this level was critical. Since a nitrogen deficient medium would limit cellulose digestion, valeric acid synthesis and/or utilization under these conditions could be investigated. The results of five experiments are combined and presented in figure 1. Firstly, the response to increased nitrogen levels was an increase in cellulose digestion and T.C.A.-N formation. Secondly, valeric acid synthesis increased and/or the rate of utilization appeared to be slower in the flasks limited in available nitrogen. As the level of nitrogen was increased to 25 or more mg. per flask there was a concomitant increase in cellulose digestion and T.C.A.-N formation; however, the amount of valeric acid present in the flasks at the end of the 30-hr. fermentations decreased. These results can be interpreted as showing that when conditions favorable to cellulose digestion were provided in the medium, the organisms, probably cellulolytic-types, utilized the valeric acid faster than it was being formed. Furthermore, it appears that valeric acid was metabolized by cellulolytic organism(s) which is in keeping with the growth promoting action for this acid reported by Bentley *et al.* (1955a).

Discussion. The disappearance of valeric acid added to the *in vitro* fermentation medium coupled with increased cellulose digestion and syn-

thesis of bacterial protein implies that valeric acid is an important metabolite for cellulolytic rumen organisms. Presumably iso-butyric acid or other 5- or 6-carbon volatile fatty acids which have been previously shown to

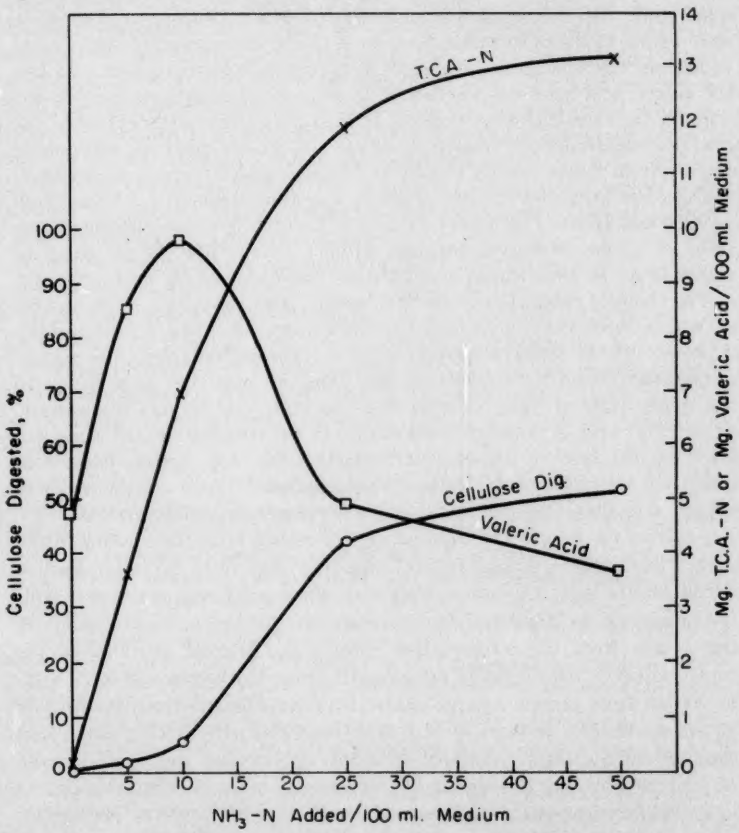

Figure 1 The effect of urea nitrogen level on protein synthesis, cellulose digestion and valeric acid content using a starch - cellulose medium. (average of 5 experiments)

have cellulolytic factor activity *in vitro* (Bryant and Doetsch, 1955; Bentley *et al.*, 1955a) may have given similar results. A study of acids other than valeric acid was not undertaken because of the inability to separate some of them with the analytical method for VFA used, for example, iso-valeric acid from valeric acid.

If starch, glucose or combinations of starch or glucose with cellulose were used as the major carbohydrate substrates, there appeared to be a net synthesis of valeric acid suggesting that the amylolytic organisms either do not require this acid for growth or that their requirement as a group is much lower than for cellulolytic organisms. When both starch and cellulose were added to the *in vitro* medium, valeric acid disappearance was related to the amount of cellulose digested, i.e., when cellulose digestion was low the valeric acid level was unchanged or increased but as conditions favorable for cellulose digestion were established the valeric acid content of the medium was reduced. Greater synthesis of valeric acid (a cellulolytic factor) from "more readily digestible" carbohydrates suggests an explanation for the increased *in vitro* cellulose digestion observed by Arias *et al.* (1951) and Hunt *et al.* (1954) with a 1:2 starch:cellulose mixture *versus* cellulose alone. Moreover, Belasco (1956) found that the inclusion of starch in an *in vitro* rumen fermentation increased valeric acid synthesis.

The valeric, iso-butyric, 2-methylbutyric, and iso-valeric acid content of rumen juice from sheep fed various rations has been determined by Annison (1954) using gas-liquid partition chromatography. The lowest values were found for animals fed hay alone whereas they were higher in the rumen juice of those animals receiving grain and protein concentrate. Shaw *et al.* (1957) found that the nature of the concentrate had a marked effect on the level of higher volatile fatty acids, e.g., rumen juice from cows fed an experimental ration which included bread contained more valeric acid than that from cows fed a lower energy-yielding ration.

Analyses for valeric and caproic acids of rumen juice from a steer fitted with a permanent ruminal fistula, indicated that by 3 hr. after feeding (good alfalfa hay) the concentration of these acids was increased. This increase could be explained by synthesis of valeric and caproic acids in the rumen from the nitrogen-free extract and protein portions of the forage as suggested by the *in vitro* results presented herein and fatty acids formation from protein (amino acids) by rumen organism as reported by El Shazly (1952). Johnson *et al.* (1957) and Dehority (1957) found that the "cellulolytic factor" activity of valine, proline and the leucines could be explained by their conversion to volatile fatty acids. A stimulatory effect of protein hydrolysates was reported by Hall *et al.* (1954). The importance of these relationships in understanding the practical application of the *in vitro* studies with 5- and 6-carbon fatty acids as "cellulolytic-factors" by Bentley *et al.* (1955) and Hungate and Dyer (1956) must be considered. Thus amino acid metabolism, fatty acid formation and carbohydrate digestion inter-relationships for the rumen microflora must be studied in greater detail both *in vitro* and *in vivo* if we are to expand our knowledge of the role of higher volatile fatty acids in rumen microorganism nutrition and activity.

Summary

The composition of the carbohydrate substrate in the *in vitro* rumen fermentation was found to influence the concentration of valeric acid present in the medium at the end of 30-hr. experiments. Little or no valeric acid was synthesized when cellulose was the carbohydrate substrate or, alternatively, the ultilization exceeded the rate of formation under these conditions. Likewise, if valeric acid was added at the start of an experiment, the final level was only 40 to 60% of the amount added initially. However, 2–10 mg. of valeric acid per 100 ml. of the *in vitro* medium was found in flasks containing a carbohydrate mixture composed of 1/3 to 1/2 starch with cellulose making up the remainder. Furthermore, valeric acid added to the starch-cellulose medium at the start of the experiment did not appear to be utilized by the micro-flora.

Rumen organisms grown in a medium deficient in urea nitrogen appeared to synthesize valeric acid; however, as the level of added nitrogen was increased the level of valeric acid in the flasks decreased. This change in valeric acid concentration was accompanied by an increase in the amount of cellulose digested and in the content of trichloroacetic acid-insoluble nitrogen in the flasks, suggesting that as microbial growth increased the rate of valeric acid synthesis decreased and/or the rate of utilization increased.

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THE RATES OF DIGESTION OF THE CELLULOSE OF SOME PLANT FRACTIONS BY RUMEN MICROORGANISMS *IN VITRO* [1]

R. L. Salsbury, A. L. VanderKolk,[2] Betty V. Baltzer and R. W. Luecke [3]

Michigan State University, East Lansing

MUCH of the recent work on the digestion of cellulose by rumen organisms has been directed toward the determination of the microbial requirements for nutrients and accessory factors. The ultimate objective has been to obtain sufficient basic data to permit the correction of deficiencies in various feeds, particularly in poor quality roughages, in order to attain maximum utilization of cellulose. To attain such a maximum, it is necessary to consider the roughage as well as the microorganisms, for there is good evidence that the extent of lignification of a roughage places a limitation upon the digestion of its cellulose. It is possible, too, that the celluloses from different sources may show differences in susceptibility to microbial attack, even in the presence of an adequate supply of nutrients.

The experiments presented here were designed to determine the relative rates of cellulose digestion of a number of plant materials when they were incubated with the fluid portion of "normal" rumen ingesta. If it can be assumed that an adequate supply of microbial nutrients was present, differences in rates of digestion should be due to inherent differences in the cellulosic materials.

Experimental

Inocula for these experiments were obtained from two permanently fistulated Hereford steers hereafter designated as Steer B and Steer C. During the early part of the experimental period, each animal received timothy hay plus two pounds of a concentrate mixture per day. Later, alfalfa hay was substituted for the timothy hay. The rate of fermentation of the reference substrate (Solka-Floc) was not measurably affected by this substitution.

Laboratory fermentations and cellulose determinations were conducted as described by Salsbury *et al.* (1956) with the following modifications: 20 ml. of the inoculum-substrate suspension were pipetted into each sac which was then suspended in 80 ml. of mineral solution. Replicates were

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[2] Present address: Michigan Department of Health, Lansing, Mich.

[3] Department of Agricultural Chemistry. The authors wish to express their appreciation to the Department of Animal Husbandry for the use of the fistulated steers.

incubated for periods of 3, 6, 9, 12, and 24 hr. This permitted the construction of curves which indicated the rates of cellulose digestion over 24-hr. periods. It was found that inocula from Steer C contained dialyzable substance(s) which caused the cellulose determinations on unincubated samples to give abnormally high values. After incubation for 3 hr. or longer the substance(s) had apparently dialyzed out of the sacs to such an extent that there was no longer any measurable interference with the cellulose determinations. As a result, the difference between the cellulose content of the unincubated controls and that of the incubated samples did not entirely represent loss due to digestion of the cellulose. Therefore, in subsequent experiments the dialysis of controls was adopted as standard procedure. This did not appreciably affect results obtained with inocula from Steer B.

The plant materials used were Solka-Floc,[4] cotton linters,[5] dehydrated alfalfa meal, wheat straw, and corn cobs. These were extracted with a 2:1 benzene:alcohol mixture and the extracted materials treated by the method of Wise *et al.* (1946) for the preparation of holocellulose and "α-cellulose". The untreated plant materials and the fractions obtained from them were then used as substrates for the laboratory fermentations.

Alfalfa meal, corn cobs, and soybean hulls were extracted with 2:1 benzene:alcohol mixture and the extracted materials used to prepare hemicelluloses by the method of Wise *et al.* (1946). No attempt was made to fractionate the hemicelluloses. Consequently, the holocelluloses were treated directly with 24% KOH rather than being given a preliminary treatment with 5% KOH. In order to determine whether the hemicelluloses had a stimulatory effect upon cellulose digestion, they were used at a level of 0.25% in fermentations in which Solka-Floc was the source of cellulose.

Results and Discussion

The rates of digestion of the cellulose in the preparations from the various plant materials are shown in table 1. When the untreated materials were used, alfalfa meal cellulose was the most rapidly digested in the first half of the fermentation period. Solka-Floc cellulose was digested at a more rapid rate later in the fermentation period but showed a slow initial rate. Extraction with benzene:alcohol (2:1) slightly increased the digestion of cellulose for all the plant materials except Solka-Floc, without appreciably affecting the relationship of one to the other.

In the comparison of the same fraction from different sources it can be seen that with each fraction the cotton linter cellulose showed the slowest rate while Solka-Floc showed a slow initial rate followed by a more rapid digestion. The cellulose digestion shown by the holocelluloses from alfalfa, wheat straw and corn cobs was faster and more complete than that shown

[4] A wood cellulose, Solka-Floc BW-40, Brown Co., Berlin, N. H.
[5] The short fibers left on the cotton seed after the removal of the staple cotton: Obtained from Buckeye Cotton Oil Co., Cincinnati 17, Ohio.

TABLE 1. RATES OF CELLULOSE DIGESTION WHEN FRACTIONS FROM VARIOUS PLANT MATERIALS WERE USED AS SUBSTRATES [a]

Substrate	Cellulose digested [b] in									
	3 Hr.		6 Hr.		9 Hr.		12 Hr.		24 Hr.	
	%		%		%		%		%	
Untreated	Av.	Range	Av.	Range	Av.	Range	Av.	Range	Av.	Range
Solka-Floc	4.5	3.7– 5.0	8.4	6.7–10.2	15.2	13.6–16.6	28.9	25.8–31.1	80.6	79.1–82.2
Cotton linters	8.3	7.2– 8.9	8.1	7.1– 9.3	8.3	6.4– 9.9	9.9	7.5–12.4	40.1	37.6–41.6
Alfalfa	15.5	14.6–17.2	25.9	24.4–28.0	28.7	26.6–32.0	37.5	36.7–38.7	46.2	45.9–46.4
Wheat straw	6.9	4.4– 9.4	15.2	13.1–17.7	18.4	17.2–19.2	22.9	20.9–25.2	39.7	37.8–40.9
Corn cobs	10.3	6.6–12.3	15.3 [c]	11.6–15.9	17.6	13.5–22.8	17.9	14.7–21.4	38.3	37.5–38.9
Extracted										
Solka-Floc	3.8	2.6– 5.1	6.7	6.0– 7.1	12.3	12.1–12.7	28.9	28.0–30.0	81.1	80.5–82.0
Cotton linters	5.6	4.5– 6.3	7.9	6.1– 9.8	10.7	9.2–11.6	15.9	14.8–16.4	49.1	45.9–51.9
Alfalfa	16.9	14.8–20.8	27.7	27.3–28.2	36.5	34.8–37.7	42.7	41.8–43.5	50.6	50.1–51.5
Wheat straw	17.2	16.8–18.1	22.6	21.9–23.3	26.8	26.4–27.4	33.4	32.8–34.2	48.2	46.9–48.9
Corn cobs	16.2	8.9–24.8	22.3	18.3–24.7	22.3	17.0–28.0	35.6	35.0–36.8	46.7	45.4–48.4
Holocellulose from										
Solka-Floc	1.9	1.3– 2.4	6.5	6.5– 6.6	17.7	15.1–19.7	35.4	32.9–38.4	82.0	81.8–82.1
Cotton linters	5.9	4.6– 6.7	5.6	4.1– 6.6	10.8	9.9–11.3	19.3	17.9–21.5	55.0	52.3–57.9
Alfalfa	14.6	12.6–16.8	25.8	24.3–26.8	45.0 [c]	44.6–45.4	54.5	52.9–55.6	79.4	78.8–79.7
Wheat straw	19.7	19.3–20.1	31.5	31.1–32.2	46.0	43.3–48.0	63.0	60.5–65.3	79.2 [d]	.....
Corn cobs	22.2	19.0–24.5	28.3	25.6–30.3	40.2	36.9–42.8	55.7	52.5–60.3	86.0	84.6–87.1
"α-Cellulose" from										
Solka-Floc	.6	0.2– 1.3	5.2	2.8– 6.9	17.0	13.6–21.1	35.8	34.6–37.9	78.8	76.6–80.1
Cotton linters	7.3	6.5– 8.6	10.7	9.5–11.5	18.2	16.5–20.1	30.0	26.8–31.9	67.2	60.2–71.4
Alfalfa	12.3	12.0–12.6	21.7	20.5–23.3	39.8	38.9–40.6	48.8	47.0–52.1	77.1	76.3–77.9
Wheat straw	12.5	11.6–13.6	19.7	15.5–23.9	36.6	35.0–38.4	56.2	52.7–58.9	85.3	84.9–85.7
Corn cobs	11.3	7.9–13.6	20.4	19.3–21.0	40.9	39.3–42.3	57.4	56.9–58.2	86.0	85.6–86.4

[a] The experiments were so designed that the untreated, extracted, holocellulose and α-cellulose fractions of any one plant material were incubated with the same inoculum.
[b] Average of 3 determinations.
[c] Average of 2 determinations.
[d] One determination.

by the original plant materials and was roughly similar for the three holocelluloses. Comparable rates were obtained when the "α-celluloses" from these materials were used and here the rates more nearly coincided, particularly those of wheat straw and corn cobs.

Huhtanen and Elliott (1956) suggest that the presence of an accessory factor for cellulose digestion may account for the fact that digestion of the cellulose in alfalfa meal is limited to a certain portion of the total apparent cellulose. In the experiments reported here, however, the similarity and rapidity of the rates of cellulose digestion for the holocelluloses and "α-celluloses" obtained from alfalfa, wheat straw, and corn cobs are supporting evidence for the view that incrusting materials are of con-

TABLE 2. EFFECT OF ADDED HEMICELLULOSES ON RATE OF CELLULOSE DIGESTION[a]

Source of hemicellulose added	Cellulose digested[b] in				
	3 Hr.	6 Hr.	9 Hr.	12 Hr.	24 Hr.
	%	%	%	%	%
None added	4.1	8.6	15.6	29.8	82.8
Alfalfa	6.6	9.1	17.0	29.6	84.9
Corn cobs	3.2	6.7	14.5	32.0	85.7
Soybean hulls	−0.6	7.6	16.0	33.3	85.8

[a] Substrate 1% Solka-Floc.
[b] Average of 3 determinations.

siderable importance in deciding the digestibility of the cellulose of roughages. On the other hand, the slow initial rates shown by the Solka-Floc and cotton linters preparations require explanation. One possibility is that the Solka-Floc and cotton linters preparations lacked some accessory factor, as suggested by Huhtanen and Elliott (1956), but that this factor was present in the roughage preparations. It appeared that hemicelluloses might constitute such a factor since they were present in quantity in the roughage holocelluloses and it is unlikely that the corresponding "α-celluloses" would be completely free of them. However, table 2 shows that the addition of hemicelluloses from alfalfa, corn cobs, or soybean hulls had no appreciable effect on the digestion of Solka-Floc cellulose. An apparent increase in digestion attributed to the addition of alfalfa hemicelluloses (Salsbury and Luecke, 1956) was found to be due, at least in part, to the dialyzable material in the inoculum from Steer C which interfered with the cellulose determination. The failure of added hemicelluloses to increase the rate of cellulose digestion is in agreement with the results reported by McBee (1953) who found that the feeding of hemicellulose did not result in an increased activity of the rumen microorganisms *in vitro* when cellulose was used as the substrate.

Consideration should also be given to the possibility that the slow initial rates shown by Solka-Floc and cotton linters indicate that the

celluloses of these materials have a different structure than those of the roughages. This might involve the presence of non-glucose units in the cellulosic chains (Norman, 1937) or a difference in the ratio of crystalline to amorphous cellulose (Siu, 1951; Preston, 1952).

Summary

The rates of digestion *in vitro* of the celluloses of a number of plant materials have been compared by incubating various fractions with strained fluid from bovine rumen ingesta. Holocelluloses and "*a*-celluloses" prepared from roughages showed more rapid and complete cellulose digestion than did the original plant materials. Addition of hemicelluloses from alfalfa, corn cobs, or soybean hulls did not increase the slow initial rate of cellulose digestion shown by Solka-Floc.

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[b] Average of 3 determinations.

siderable importance in deciding the digestibility of the cellulose of roughages. On the other hand, the slow initial rates shown by the Solka-Floc and cotton linters preparations require explanation. One possibility is that the Solka-Floc and cotton linters preparations lacked some accessory factor, as suggested by Huhtanen and Elliott (1956), but that this factor was present in the roughage preparations. It appeared that hemicelluloses might constitute such a factor since they were present in quantity in the roughage holocelluloses and it is unlikely that the corresponding "α-celluloses" would be completely free of them. However, table 2 shows that the addition of hemicelluloses from alfalfa, corn cobs, or soybean hulls had no appreciable effect on the digestion of Solka-Floc cellulose. An apparent increase in digestion attributed to the addition of alfalfa hemicelluloses (Salsbury and Luecke, 1956) was found to be due, at least in part, to the dialyzable material in the inoculum from Steer C which interfered with the cellulose determination. The failure of added hemicelluloses to increase the rate of cellulose digestion is in agreement with the results reported by McBee (1953) who found that the feeding of hemicellulose did not result in an increased activity of the rumen microorganisms *in vitro* when cellulose was used as the substrate.

Consideration should also be given to the possibility that the slow initial rates shown by Solka-Floc and cotton linters indicate that the

celluloses of these materials have a different structure than those of the roughages. This might involve the presence of non-glucose units in the cellulosic chains (Norman, 1937) or a difference in the ratio of crystalline to amorphous cellulose (Siu, 1951; Preston, 1952).

Summary

The rates of digestion *in vitro* of the celluloses of a number of plant materials have been compared by incubating various fractions with strained fluid from bovine rumen ingesta. Holocelluloses and "α-celluloses" prepared from roughages showed more rapid and complete cellulose digestion than did the original plant materials. Addition of hemicelluloses from alfalfa, corn cobs, or soybean hulls did not increase the slow initial rate of cellulose digestion shown by Solka-Floc.

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THE EFFECT OF CERTAIN INOCULATIONS ON THE UTILIZATION OF UREA OR BIURET BY GROWING LAMBS[1]

R. C. EWAN,[2, 3] E. E. HATFIELD AND U. S. GARRIGUS

Illinois Agricultural Experiment Station[4]

UREA can be utilized as a nitrogen source for ruminants, but the extent of its use is limited because of possible toxic effects. Biuret, a condensation product of urea, has been shown to be less toxic to animals than urea. Meiske *et al.* (1955) demonstrated that urea produced toxic symptoms and death while the same and higher levels of biuret were not toxic. Hatfield *et al.* (1956) have shown that relatively high levels of biuret administered as a drench or in feed produced no definite toxicity. This work has also been substantiated by the work of Berry *et al.* (1956).

Studies of the utilization of biuret *in vivo* have indicated that it can be used as a nitrogen source for ruminants even though Belasco (1954) using an artificial rumen found that biuret as a nitrogen source gave only 7% as much cellulose digestion as when urea was the nitrogen source. Meiske *et al.* (1955) found that lambs utilized the nitrogen from biuret as well as the nitrogen from urea when gains and feed efficiency were the criteria. Gaither *et al.* (1955) have reported nitrogen balance studies with lambs which showed that the nitrogen of biuret was retained as well as the nitrogen from urea. Several other workers have reported significant utilization of biuret nitrogen (Campbell *et al.*, 1956; Welch *et al.*, 1956; Berry *et al.*, 1956; Hatfield *et al.*, 1955).

The purpose of this study was to determine indirectly whether microorganisms which utilize urea were the same as those that utilize biuret.

Experimental Procedure and Materials

Fifty-four fine-wooled, southwestern lambs were used in this experiment. They were maintained on alfalfa hay until they were started on experiment. The lambs were fed the basal ration shown in table 1, which was designed to meet the lamb's nutrient requirements except for nitrogen, during a preliminary period of at least 20 days. After the preliminary period, nine lambs were selected for the metabolism trial on the basis of equal food consumption. These lambs were followed in service by five other sets of nine lambs each.

[1] This work was financed in part by a grant-in-aid from the Grace Chemical Company, Memphis, Tennessee. Aid was also received from Moorman Manufacturing Company, Quincy, Illinois, which supplied the mineral mixture, courtesy of Arthur Nesbit.

[2] This research completed in partial fulfillment of the requirements for M.S. Degree in Animal Science, University of Illinois, Urbana, June, 1957.

[3] Presently on active duty with the U. S. Air Force.

[4] Department of Animal Science, University of Illinois, Urbana.

The design of the metabolism trial was a randomized complete block. Three nitrogen sources (1) basal, (2) the basal plus 2.2% urea, and (3) the basal plus 2.7% biuret (determined by solubility test to be essentially pure biuret) were used and three inoculation treatments—(1) none, (2) the rumen contents from sheep maintained for 20 months on a similar basal ration with urea added, or (3) the rumen contents from sheep maintained for 20 months on a similar basal with biuret added—were given in all combinations resulting in nine treatments.

TABLE 1. THE COMPONENTS OF THE BASAL RATION

	%
Corn	40.0
Dehydrated cobs	25.0
Starch	25.0
Complete mineral[a]	5.0
Molasses	3.3
Corn oil	1.0
Vitamins A and D concentrate[b]	0.3
Aurofac[c]	0.3
Choline chloride	0.1
B vitamins[d]	1.284 gm. per cwt.
Alpha-tocopherol	300.0 mg. per cwt.

[a] The mineral mixture was composed of the following in grams: $CaSO_4 \cdot 2H_2O$, 3541; $CaHPO_4 \cdot 2H_2O$, 8399; NaCl, 8204; $MgSO_4 \cdot 7H_2O$, 6819; $FeSO_4 \cdot 7H_2O$, 681; $MnSo_4 \cdot 5H_2O$, 87; $ZnSO_4 \cdot 7H_2O$, 15; $CuSO_4 \cdot 5H_2O$, 14; KI, 11; CaF, 6; $CoSO_4 \cdot 7H_2O$, 4; and $NaMoO_4 \cdot 2H_2O$, 4.

[b] The concentrate contained 3,500 USP Units of Vitamin A acetate per gram and 227,000 USP Units of Vitamin D per gram.

[c] The Aurofac contained 1.8 gm. chlortetracycline and 1.8 mg. Vitamin B_{12} per pound.

[d] The B-Vitamins added were as follows in mg. per cwt.: thiamine HCl, 50; riboflavin, 140; niacin, 500; calcium pantithenate, 450; pyridoxine HCl, 60; folic acid, 40; menadione, 40; and biotin, 4.

Inoculation samples were obtained by use of a tube into the rumen which was attached to a filter flask and a hand vacuum pump. The flask was immersed in warm water and the liquid contents of the rumen pumped out. The rumen liquor was strained to remove large particles and 50 to 100 ml. were given to the lamb as a drench. Immediately following the inoculation the lambs were placed in metabolism cages and given the experimental ration.

The collection period began four days after the inoculation treatment and was continued for a period of six days. All lambs received 800 gm. of the test ration per day. The metabolism cages were similar to those described by Bratzler (1951) which collect urine and feces quantitatively. Urine was preserved by adding 5 ml. of 10% HCl to the collection bottle and was filtered as it entered the collection bottle. Urine was collected daily and diluted to a constant volume. A 10% aliquot was saved and added to the previous day's aliquot to obtain samples for analysis. Feces were weighed daily and 10% aliquots were saved for analysis. The combined 6-day aliquot was dried in a cabinet dryer at 110° F. for 72 hours.

TABLE 2. AVERAGE COMPOSITION OF THE RATIONS DETERMINED BY ANALYSIS AND THE STANDARD ERRORS OF THE AVERAGES

Ration	Nitrogen	Dry matter	Organic matter
	%	%	%
Basal	0.72(0.024)	94.66(0.218)	88.31(0.218)
Basal plus urea	1.86(0.026)	94.60(0.255)	89.60(0.244)
Basal plus biuret	1.86(0.024)	94.80(0.252)	89.56(0.345)

The sample was then allowed to stand at room temperature for 24 hours, weighed, and ground finely in a Wiley mill for analysis. Feed and ort samples were dried and ground in the same manner.

Feed, ort, and feces samples were analyzed for nitrogen, dry matter, and ash content by the methods described by the A.O.A.C. (1950). The percentage of organic matter was calculated by subtracting the percentage of ash from the percentage of dry matter. Urine samples were analyzed for nitrogen by the same methods.

Statistical analyses of the data were by the methods of Federer (1955).

Results and Discussion

The average composition of the experimental rations as determined by analysis may be found in table 2. The basal ration contained an average of 4.5% protein equivalent while the basal supplemented with urea or biuret contained 11.6% protein equivalent. An average of 61.3% of the nitrogen in the supplemented rations was supplied by urea or by biuret.

Table 3 gives the apparent digestion coefficients and the nitrogen balance data. Apparently digestion coefficients for dry matter, nitrogen, and organic matter were all improved ($P<0.01$) by the addition of nitrogen to the

TABLE 3. SUMMARY OF THE DIGESTION COEFFICIENT AND NITROGEN BALANCES

Ration	Basal			Basal plus urea			Basal plus biuret		
Inoculation	None	Urea	Biuret	None	Urea	Biuret	None	Urea	Biuret
Nitrogen digestion coefficient, %	28.33	32.79	26.25	72.77	72.89	70.98	69.51	67.89	70.98
Standard error	18.07	13.67	13.97	4.11	5.80	3.47	3.33	5.24	3.52
Nitrogen balance 61day period, gm.	−2.71	−1.12	−2.13	24.54	21.74	15.33	11.03	10.77	18.54
Standard error	6.77	6.18	4.67	6.75	7.34	7.93	6.30	6.25	3.68
Nitrogen intake retained, %	−9.17	−5.84	−9.06	29.51	26.81	18.37	14.19	15.71	22.85
Standard error	20.14	21.39	17.76	6.31	9.54	9.27	7.93	8.54	3.43
Dry matter digestion coefficient, %	60.02	63.30	61.59	71.81	72.85	74.04	70.55	67.57	69.86
Standard error	5.95	5.62	6.01	2.60	5.47	3.79	4.51	3.08	1.99
Organic matter digestion coefficient, %	61.18	64.22	62.91	73.20	73.97	75.46	71.93	70.97	71.20
Standard error	5.64	5.81	5.30	2.63	5.63	5.49	4.28	2.82	2.46

ration as was the N balance when expressed as a percent of nitrogen intake. Dry matter and organic matter digestion was slightly lower when biuret was the nitrogen source than when urea was used. This difference was not significant. Welch *et al.* (1956) have reported that rations containing biuret fed to lambs had lower organic matter and nitrogen digestion coefficients than rations containing urea. This may indicate that the microorganisms that utilize biuret are not as efficient at promoting the digestion

TABLE 4. ANALYSIS OF VARIANCE OF NITROGEN BALANCES

Source of variation	Degrees of freedom	Mean square	Variance ratio	Probability less than
Replicates	5	12.365	.....	N.S.
Treatments	8	660.434	15.170	0.01
Rations				
Basal versus urea and biuret	1	4323.264	99.258	0.01
Urea versus biuret	1	453.051	10.402	0.01
Inoculation treatment within rations				
Basal ration				
None versus urea and biuret	1	4.709	.....	N.S.
Urea versus biuret	1	1.013	.....	N.S.
Basal plus urea				
None versus urea and biuret	1	143.640	.....	N.S.
Urea versus biuret	1	124.485	.....	N.S.
Basal plus biuret				
None versus urea and biuret	1	52.563	.....	N.S.
Urea versus biuret	1	181.119	4.158	0.05
Error	40	43.556	.....	....
Total	53	.....	.....	....

of organic matter as urea utilizing organisms. There were no differences in these measures that were due to inoculation treatment.

The nitrogen balance data, when analyzed statistically (table 4), showed that the balance was improved ($P<0.01$) when nitrogen was added to the ration. When urea was added to the ration, the balance was higher ($P<0.01$) than when biuret was the nitrogen source. There were no differences due to inoculation except when the lamb receiving the inoculation treatment from sheep maintained on a ration containing biuret was given the ration containing biuret. Under these conditions the nitrogen balance was improved ($P<0.05$) and since the standard error was smaller, this appears to be a fairly uniform response. This difference seems to indicate that the microorganisms that utilize biuret were supplied by the inoculation treatment and that these microorganisms were not present in large numbers in the rumen of uninoculated lambs.

This trend has also been shown in a growth study comparing urea and biuret (Hatfield *et al.*, 1956). Lambs were fed a semi-purified, low-nitrogen ration supplemented with urea or with biuret. The lambs fed urea as the major nitrogen source outgained those receiving the same ration supple-

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This trend has also been shown in a growth study comparing urea and biuret (Hatfield *et al.*, 1956). Lambs were fed a semi-purified, low-nitrogen ration supplemented with urea or with biuret. The lambs fed urea as the major nitrogen source outgained those receiving the same ration supple-

mented with biuret during the first period. In latter periods, however, the gains of the two groups were similar. When biuret is fed, an adaptation period seems to be needed before the microorganisms are able to utilize the nitrogen efficiently. These data may also explain why Belasco (1954) obtained a low response (using cellulose disappearance as a criterion of nitrogen utilization) when biuret was used in the artificial rumen.

Summary and Conclusions

A randomized complete block experiment with six replicates was conducted using 54 lambs. The study compared the effect of three nitrogen sources (1) basal, (2) basal plus urea, and (3) the basal plus biuret, and three inoculation treatments (1) none, (2) rumen contents from sheep maintained on a similar ration containing urea and (3) rumen contents from sheep maintained on a ration containing biuret, in all combinations on nitrogen balance, percentage of nitrogen intake retained, nitrogen digestion, organic matter digestion, and dry matter digestion.

The data seem to justify the following conclusions: 1. All measures were improved ($P<0.01$) by the addition of nitrogen to the ration. 2. The apparent digestion coefficients for organic matter and dry matter were depressed when the basal plus biuret was compared to the basal plus urea. This difference was not significant, however. 3. Nitrogen balances were higher ($P<0.01$) when the basal plus urea was compared to the basal plus biuret. 4. Nitrogen balance was higher ($P<0.05$) when lambs were fed the biuret supplemented ration and received the inoculation treatment with rumen contents from sheep on a ration containing biuret when compared to either of the other inoculation treatments. No other effects were observed from inoculation treatments.

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FEEDLOT BLOAT STUDIES. III. RUMEN FATTY ACIDS AND METABOLIC ACTIVITY OF RUMEN BACTERIA OF ANIMALS PLACED ON A BLOAT-PRODUCING DIET [1, 2]

DON R. JACOBSON, IVAN L. LINDAHL,[3] J. J. MCNEILL, J. C. SHAW, R. N. DOETSCH AND R. E. DAVIS [3]

University of Maryland, and USDA [4]

VERY little research has been conducted on the relationship between the rumen microflora and bloat. It was suggested in the review of Cole *et al.* (1945) that an abnormal rumen microflora might be the cause of bloat. Koffman (1937) reported a decrease in a number of large forms of protozoa in the ingesta of bloated sheep, and Quin (1943a; 1943b) has postulated that the presence of characteristic pseudo-yeast cells is a factor in bloat.

The purpose of this study was to obtain information regarding the metabolic activity of rumen bacteria prior to and during the developmental stages of feed-lot frothy bloat. In addition, it was hoped that this study would provide some information as to the validity or proper interpretation of *in vitro* dissimilations as indicators of the reactions taking place in the rumen.

Experimental Plan and Methods

Much of the experimental plan is given in detail in previous papers (Lindahl *et al.*, 1957; Jacobson, *et al.*, 1957), including the experimental conditions in regard to the animal phases and the method of estimating the incidence of bloat (bloat index). Seven steers and heifers (800–1000 lb.) were employed for a 16-week period. In general, rumen samples were taken at week 0, at which point the animals had been on a diet of corn silage and mixed grass hay *ad libitum* for eight weeks, at week 4 (after four weeks on 5 lb. of mixed grass hay and 6 lb. of bloat-producing mixture), and at weeks 7, 8, 9, 10, 11, 12, 13 and 16 at 4.5 hours after feeding.

Biochemical Analyses of Strained Rumen Fluid. Each rumen sample was checked qualitatively for relative concentration of iodine staining substance (ISS) using a spot plate technique (Robinson *et al.*, 1955). The intensity of the blue color that resulted was arbitrarily rated 0, 1, 2, 3, or 4. This method gives a rough qualitative evaluation of the amount of starch-like polysaccharide stored within the bacterial cells.

[1] Scientific Article No. A661, Contribution No. 2860 of the Maryland Agricultural Experiment Station. Taken from a thesis presented by Don R. Jacobson to the Graduate School, University of Maryland, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Dairy Husbandry, University of Maryland.

[2] This work was supported in part by Animal Disease and Parasite Research Division, A.R.S., U.S.D.A.

[3] Animal Husbandry Research Division, A.R.S., U.S.D.A., Beltsville, Md.

[4] Dairy and Microbiology Departments, Maryland Agricultural Experiment Station, College Park.

The presence or absence of reducing substances was determined with Benedict's qualitative reagent. Lactic acid in the rumen fluid was analyzed by the method of Barker and Summerson (1941). Rumen samples were analyzed for volatile fatty acids by the method of Keeney (1955).

Metabolic Studies of Ruminal Microorganisms. The cell suspension technique of Doetsch *et al.* (1955) was employed. Dissimilation studies on glucose and cellobiose were done by mixing 5 ml. of the above standardized cell suspension, 2 ml. of either glucose or cellobiose solution (300 uM per ml.) and 3 ml. of M/15 phosphate buffer (pH 6.8) and then incubated at 39° C. under an atmosphere of oxygen-free nitrogen for 24 hours. An endogenous control was used for each rumen sample in which 2 ml. of buffer solution were added instead of 2 ml. of substrate. The ISS, lactic acid, and volatile fatty acid analyses were determined on the fermentation liquors in the same manner as on the rumen fluid. A qualitative check for residual substrate in each fermentation liquor after 24 hours incubation was done in the same manner as the analyses for reducing substances in the rumen fluid.

Results

Volatile Fatty Acids in the Rumen and Their Production from Glucose and Cellobiose in Vitro. For each rumen sample taken a portion was set aside from which the bacteria were obtained for dissimilation studies. In this manner comparisons between fatty acid values in the rumen and fatty acid values resulting from glucose and cellobiose dissimilations were made.

The results of fatty acid analyses of rumen fluid are given in figure 1. The corresponding results for *in vitro* glucose and cellobiose dissimilations are given in figures 2 and 3. The amount of each fatty acid arising from dissimilation was obtained by subtracting the endogenous amount from the total amount of each acid present at the end of 24 hours incubation and dissimilation. The molar percentage of each acid was then calculated. It is recognized that this correction may not be a completely precise procedure, but it may be employed in routine work of this nature. It will be noted in figure 1 that there was some increase in the concentration of the total volatile fatty acids in the rumen during the early weeks of the experiment, after the diet had been changed from one of hay and corn silage to hay and 6 lb. of bloat-producing mixture daily. A further and much greater increase took place following a complete changeover to the bloat-producing diet. As will be noted from figures 2 and 3, there was also a very large increase in the production of total volatile fatty acids from the bacterial dissimilation of glucose and cellobiose. A rather remarkable parallel was noted in the total volatile fatty acid production resulting from the dissimilation of these two substances by bacterial suspensions from the same samples of rumen fluid.

The changes in the molar percentage of each volatile fatty acid in the rumen contents are also shown in figure 1. With the exception of the original

values, acetic acid and propionic acid exhibited an inverse relationship throughout the entire experimental period. Acetic acid gradually decreased and then increased toward the end of the experiment, whereas propionic

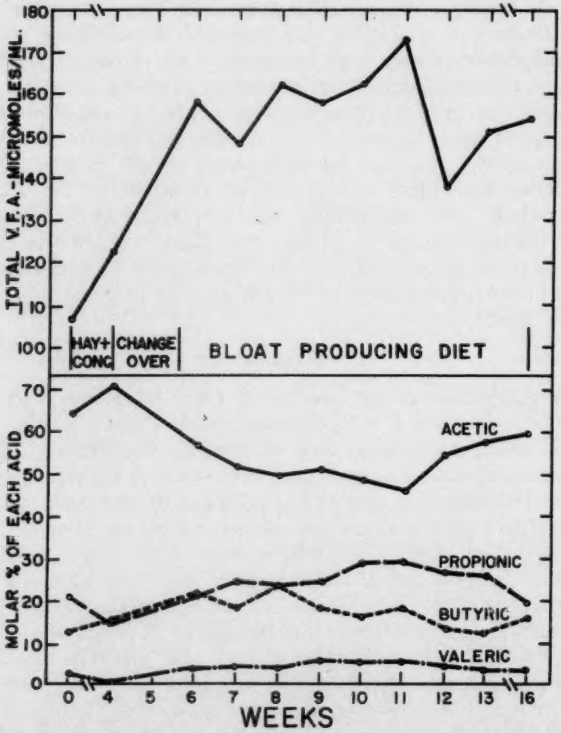

Figure 1. The effect of the bloat producing diet on rumen volatile fatty acids. Each value represents an average of 4 animals. Microbial dissimilations from these rumen samples are shown in Figures 2 and 3.

acid increased and then decreased toward the end. The differences in both acetic acid and propionic acid at week 11 compared to week 16 of the experiment are statistically significant ($P<0.05$). The percentage of butyric acid increased and then decreased whereas that of valeric acid did not show much change until after the fourth week when some increase occurred.

The interesting inverse relationship between propionic acid and acetic acid which was observed in the rumen contents will also be noted in the bacterial dissimilations of glucose and cellobiose, but to an accentuated degree. Once again, there was a remarkable agreement between the

glucose and cellobiose dissimilations (figures 2 and 3). The molar percentages of the fatty acids produced by these dissimilations changed when the diet fed to the animals was changed to the bloat-producing diet. The molar percentage of acetic acid decreased and the molar percentage of

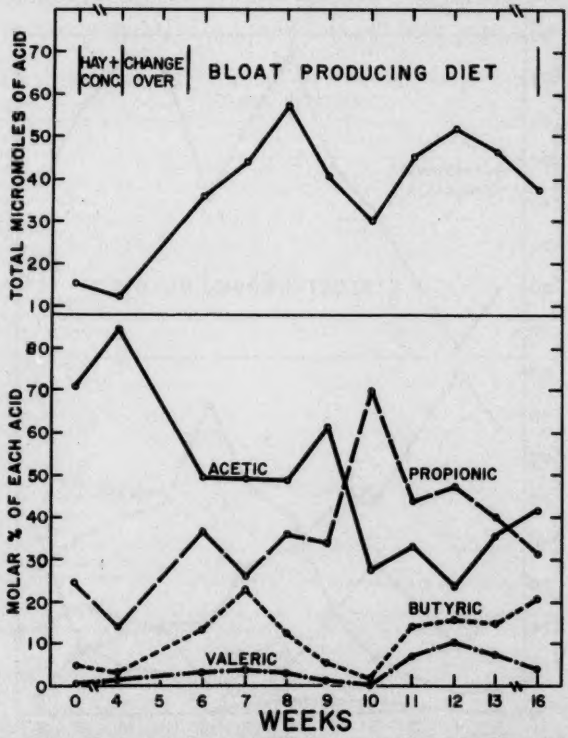

Figure 2. The effect of the bloat producing diet on volatile fatty acids which resulted from rumen microbic dissimilation of 48 micromoles of glucose. Each value represents an average of 4 animals. The cell suspensions were prepared from the same rumen samples represented in Figure 1.

propionic acid increased, both changes being quite marked, followed by the reverse for each acid, so that at the end of the period the molar percentage of each acid was similar to that when the animals were first placed on the bloat-producing diet. The molar percentage of butyric acid and especially the increases in valeric and butyric acids produced by the dissimilations toward the end of the experimental period are particularly interesting. Since the results of the dissimilation of glucose closely parallel the results obtained from the dissimilation of cellobiose, it must be concluded that these data represent changes in bacterial activity.

Whereas seven animals were employed in the 16-week experiment, the

data from only four were included in figures 1, 2 and 3 because one animal went off feed and the other two animals, which were fistulated, yielded values which were often far outside the range of values for the non-fistulated animals. For example, at week 4, in the case of one of the fistulated animals, the total fatty acids (micromoles per ml. of rumen

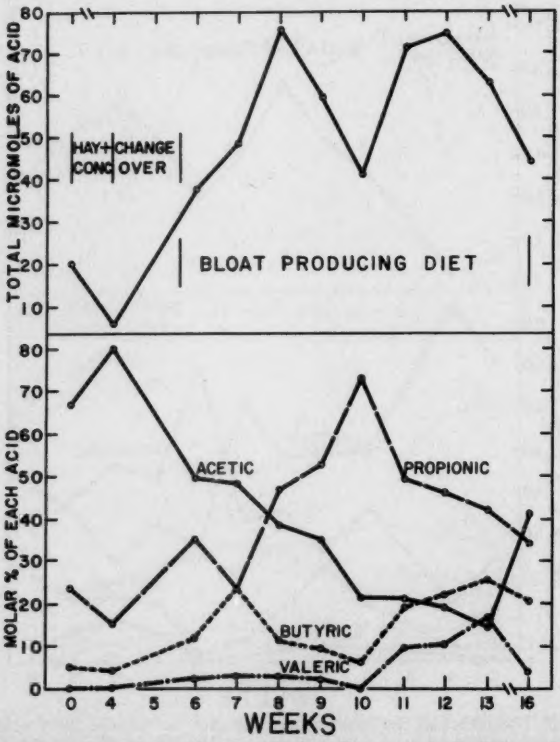

Figure 3. The effect of the bloat producing diet on volatile fatty acids which resulted from rumen microbic dissimilation of 48 micromoles of cellobiose. Each value represents an average of 4 animals. The cell suspensions were prepared from the same rumen samples represented in Figure 1.

fluid) produced from the dissimilation of glucose and cellobiose was 56 and 67 uM, respectively, whereas the values were only 11 and 6, respectively, for the four animals which were not fistulated and which exhibited good appetites throughout the entire experimental period. In addition, the molar percentage of each acid was unusual, the values from cellobiose dissimilation being as follows: acetic —25.3, propionic —0.0, butyric —66.2, valeric —.84, and higher acids —0.0. Similar values were obtained

from the dissimilation of glucose by bacteria obtained from the same rumen samples.

In the case of the animal which went off feed during the early part of the experiment, the molar percentages of fatty acids in the rumen were about the same as those of the other animals. However, the values resulting from the dissimilation of glucose and cellobiose were altered greatly.

TABLE 1. THE EFFECT OF DIET ON CERTAIN SUBSTANCES IN THE RUMEN CONTENTS [a]

Determination	Week of Experiment										
	0[b]	4[c]	6[d]	7	8	9	10	11	12	13	16
Relative concentration of ISS in rumen liquor	0.0	0.8	1.5	3.0	0.8	1.5	0.5	1.0	...	2.0	2.8
Presence of reducing substance in rumen liquor	...	...	...	...	...	...	...	...	...	...	...
Lactic acid, uM/ml. of rumen liquor	...	...	0.3	0.1	0.2	0.1	0.3	0.3	0.3	0.3	0.3
Relative concentration of ISS from glucose dissimilation in vitro	3.0	2.8	3.5	2.5	0.0	2.8	2.0	2.3	2.8	3.0	2.8
Lactic acid, uM/ml. from dissimilation of 48 uM/ml. of cellobiose	...	3.8	3.0	1.9	1.8	4.4	3.4	2.3	0.2	2.5	2.4
Relative concentration of ISS from cellobiose dissimilation in vitro	3.0	3.0	3.5	3.3	2.3	3.3	3.3	3.3	4.0	3.8	3.3
Lactic acid, uM/ml. from dissimilation of 48 uM/ml. of cellubiose	...	3.6	3.4	3.0	4.0	4.4	4.2	3.5	2.8	2.5	3.4
Total bloat index of the animals during the week of sampling	0	0	6	18	7	15	18	18	31	47	52

[a] Each value represents an average of 4 animals.
[b] Original samples taken when animals had been on corn silage and mixed grass hay ad libitum for 8 weeks.
[c] Animals sampled after 4 weeks on 5 lb. mixed grass hay and 6 lb. bloat producing mix per day.
[d] Samples taken 4 days after complete changeover to the bloat producing diet.

The molar percentages were as follows: acetic —0.0, propionic —10.5, butyric —57.5, valeric —15.4, and higher acids —16.6% Cellobiose dissimilation gave a similar distribution.

Iodine Staining Substance, Reducing Substance, and Lactic Acid. The relative concentration of an iodine staining substance (ISS) in the rumen liquor, and that resulting from the dissimilation of 600 uM of glucose and cellobiose *in vitro* are shown in table 1. The ISS was absent at week 0 when the animals were on a hay and silage ration fed *ad libitum*. However, after the animals were placed on the bloat-producing diet a considerable amount of this material was stored by the microorganisms. The concentrations of ISS resulting from the dissimilation of 600 uM of glucose and cellobiose *in vitro* was consistently high. This indicates that rumen microorganisms store ISS when a readily available source of energy is present, thus confirming the report of Gibbons *et al.* (1955). On the average, cellobiose dissimilation led to greater ISS concentrations.

Benedict's qualitative test for reducing sugars in the rumen liquor were consistently negative (table 1). The same test employed to determine the relative concentrations of the residual substrate (glucose and cellobiose) indicated that glucose was almost entirely dissimilated whereas cellobiose was not. Apparently an excess of cellobiose was present for complete utilization under the test conditions. Lactic acid (table 1) was always present in significant amounts in the rumen fluid at 4.5 hours after feeding when the animals were on the high-energy, high-concentrate, bloat-producing diet. Proportionately, the lactic acid values as compared to total volatile fatty acids in the fermentation liquor from glucose and cellobiose dissimilations were much higher than in the rumen fluid. A positive relationship was not discernible between the concentrations of the individual fatty acids, lactic acid, or ISS in the fermentation liquor, or in the rumen fluid, and the incidence of bloat in animals on the same diet.

Discussion

When the diet fed to the animals was altered from corn silage and mixed grass hay ***ad libitum*** to 5 lb. of the same lot of mixed grass hay and 6 lb. of the bloat-producting mixture, distinct changes resulted in both the total volatile fatty acid concentration in the rumen and the molar percentage of each acid. The increase in total acid concentration in the rumen was undoubtedly due to the addition of concentrate to the daily ration.

Not only did the molar percentages of the rumen fatty acids change with changes in the diet, but after the animals were placed on a constant intake (14 lb. daily) of the bloat-producing diet the molar percentages of rumen fatty acids continued to change over the remaining 74 days of the experiment.

It would appear that following certain marked changes in the diet, the physiological adjustment of the rumen microflora to the new environment may be slow. Qualitative or physiological changes in the rumen microflora may take place during adjustment to certain dietary changes as indicated by analyses of the rumen fluid and by the ***in vitro*** dissimilations of glucose and cellobiose.

While the animals were on the bloat-producing diet, changes in the concentrations of acetic and propionic acids in the rumen fluid were in the same direction but not of the same magnitude as fatty acid production ***in vitro***. Therefore, it may be postulated that under carefully controlled conditions small changes in the percentage of acetic and propionic acids in the rumen represent much greater changes in actual production of these acids. In addition, the net energy values of the feedstuffs involved very likely change during the rumen bacterial adjustment period when fatty acid production varies.

The implications of the above mentioned changing rumen bacterial metabolism on a given diet are far reaching. A major portion of past

research and practically all digestibility trials have allowed only a few days for the animals to adjust to new feeds. From the point of view of bacterial activity, it is entirely possible that different pre-fed diets could lead to an altered rumen microflora which in turn could lead to differences in digestibility. Therefore, the digestibility of a specific ration may depend upon the specific rumen microflora present at the time of the trial. In two recent reports (Lloyd *et al.*, 1956; Nicholson *et al.*, 1956) it has been shown that the length of the preliminary feeding period does have an effect on the digestion coefficients. This was particularly true where the ratio of roughage to concentrate was altered markedly. However, it remains to be shown that changes in digestibility with time on a given diet are due to an altered rumen microbial activity, though it would appear to be the case.

The animal which went off-feed had an essentially normal fatty acid ratio in the rumen. However, the corresponding fatty acid production *in vitro* was quite unusual. This poses the interesting possibility that an animal off-feed may have an "abnormal" bacterial metabolism, though it is not clear as to whether the change would proceed or follow the development of inappetence. It also suggests that even though total fatty acid production in the rumen is altered, the molar percentages of the fatty acids present in the rumen may remain within the normal range.

Summary

The concentration of volatile fatty acids increased from approximately 110 to 150 uM per ml. of rumen fluid when the animals were changed from a hay-silage diet to a bloat-producing diet. The ratio of short chain rumen volatile fatty acids changed with each diet fed and with time in the case of the bloat-producing diet. Acetic acid decreased from 55 to 46 molar percent and propionic acid increased from 21 to 29 molar percent of the total volatile fatty acids in the rumen at 4 and 39 days, respectively, on the bloat-producing diet (average of 4 animals). The trend then reversed and acetic acid increased and propionic acid decreased to 60 and 20 molar percent, respectively, after the animals had been on the bloat-producing diet for 74 days. Reducing substances were never found in the rumen liquor, whereas lactic acid was nearly always found in the rumen samples taken 4.5 hours after feeding the bloat-producing diet.

The dissimilation of glucose and cellobiose by rumen microorganisms removed from the rumen 4.5 hours after feeding showed that the proportion of the fatty acids produced changed with the diet. Following the changeover to the bloat-producing diet, the proportions of volatile fatty acids from the dissimilations were altered in an accentuated manner in the same direction as the rumen fluid. This indicates that as the individual fatty acid production in the rumen changes, the relative concentration in the rumen changes in the same direction and that relative concen-

tration in the proportions of rumen volatile fatty acids may represent relatively large changes in their production. There were striking similarities in the volatile fatty acid proportions which resulted from the cell suspension dissimilations of glucose and cellobiose.

The proportions of the fatty acids produced by *in vitro* dissimilations changed with each diet, and during the 74 days when the animals were fed the bloat-producing diet, were changing constantly. Valeric acid was apparently synthesized from carbohydrate in unusually large quantities by rumen bacteria.

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EFFECT OF SEVERAL LEVELS OF FURAZOLIDONE IN SWINE RATIONS [1]

GEORGE W. VANDER NOOT

New Jersey Agricultural Experiment Station

SINCE economical swine production depends upon rapid growth and optimal feed efficiency, new additives for swine rations are continuously sought and evaluated. Several antibiotics are widely accepted and used as growth stmiulants and antimocrobial agents (Maddock and Brackett, 1956).

Another group of agents are the nitrofurans, whose antimicrobial activity was first noted by Dodd and Stillman (1944). Main (1947) reported that nitrofurans are synthesized from agricultural waste products, such as corn cobs and oat hulls. The nitrofurans are unrelated to antibiotics, but are active against both Gram-positive and Gram-negative bacteria (Dodd, 1946). Several nitrofurans have proven valuable therapeutically and prophylactically in veterinary medicine. Gardner and Wittorff (1954) and Fangauf and von Barloewen (1954) reporting on dairy calves and poultry, respectively, noted that low level feeding of nitrofurazone decreased death loss and promoted weight gains. Guthrie (1952) reported no toxic effect in swine when they received 150–200 mg. of nitrofurazone per kg. of body weight. The drug has been successfully used against coccidiosis caused by *Eimeria deliecki* (Deom and Mortelmans, 1954). Nitrofurazone was found to be effective against necrotic porcine enteritis caused by Salmonella choleraesius (Guthrie, 1952; Lannek and Brag, 1954; Felgate and Swann, 1956). Furazolidone (Furoxone), another nitrofuran derivative, has been reported by Smith (1954) to be highly effective in poultry against various Salmonellae and to have growth-stimulating properties in poultry (Libby and Schaible, 1955; Berg *et al.*, 1955).

The purpose of this study was to determine the effect of various levels of Furoxone [2] in a swine ration for (1) growth (2) toxicity and (3) palatability.

Procedure

Purebred Duroc pigs were used throughout the two trials and were weighed individually at the start and termination. Group weights were recorded at 2-week intervals. The groups were self-fed grain, hand-watered, and housed in a barn with outside paved runways.

[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Animal Husbandry, New Brunswick.

[2] Furazolidone [N-(5-nitro-2-furfurylidone)-3-amino-2-oxazolidone], available as Furoxone concentrate veterinary, was supplied through the courtesy of the Eaton Laboratories, Norwich, New York.

The basal ration consisted of 58.4, 22.0, 7.5, 5.0, 5.0, and 2.0% of corn, barley, soybean oil meal, meat scraps, alfalfa meal, and minerals, respectively. It included 45.3 gm. of vitamin mixture [3] and 40.77 gm. of Profactor B_{12}.[3] The mineral supplement contained 0.75 lb. NaCl, 0.50 lb. ground limestone, 0.50 lb. steamed bone meal, and 0.25 lb. "mico" trace mineral mixture [4] per ton of feed. The ingredients of the rations were mixed together in a horizontal power mixer. From each batch of mixed feed, a sample was drawn from the top and bottom to determine by analysis the distribution of the additive throughout the mixture.

In the spring of 1956, 60 weanling pigs were randomly assigned to 10 groups of 6 pigs each. The groups were randomly paired into five treatment groups and each pair received a basal ration containing either 5, 10 or 15 gm. of Furoxone per ton. One treatment group received only the unmedicated basal ration, and the remaining one received 18 gm. of chlortetracycline (as Aurofac 10) per ton of basal feed.

In the second trial, conducted in the fall of 1956, 72 pigs were assigned randomly first into 12 groups of six and then paired into six treatment groups. Four of the groups received Furoxone at 5, 7.5, 10, and 50 gm. per ton of basal ration, respectively; the 12 positive controls received 18 gm. of chlortetracycline per ton; and the pigs of the sixth replicate which served as negative control received the basal ration only.

Results and Discussion

The average daily gains and feed efficiency for trial 1 are shown in table 1. Statistical analysis of the growth data failed to show that the difference between the rations, utilizing various levels of Furoxone and chlortetracycline was significant at the 5% level of probability. However, the trend in the growth results favored those groups receiving either 10 or 15 gm. of Furoxone per ton, both exceeding the negative and positive control by 2.4 and 3.3%, respectively, in average daily gains in weight. In this trial the negative control had an 0.8% greater average daily gain, and required 2.13% less feed for each pound of gain than did the chlortetracycline-fed group. The feed conversion of the group fed the 10 gm. level of Furoxone was superior to the negative and positive controls by 0.8 and 2.2%, respectively. Those fed the 15 gm. level of Furoxone utilized 0.9 and 1.21% less feed than the negative or positive control group for each pound of gain in weight.

In the second trial, the chlortetracycline group had the highest average daily gain and the greatest feed efficiency. The feed efficiency of the Furoxone-fed groups was erratic, as the groups fed 7.5 and 10 gm. levels

[3] Fortified vitamix 2-49C, Profactor 10, and Aurofac 10 were supplied through the courtesy of the American Cyanamid Company, Lederle Laboratories Division, Pearl River, New York. The vitamix 2-49C contains a minimum of 2,000 mg. Riboflavin, P.A. 4,000 mg. Niacin, 9,000 mg. Choline HCl, 10,000 mg. Folic Acid, 60 mg. per pound, respectively. Profactor 10 contains 10 mg. of B_{12} per pound.

[4] "Mico" trace minerals contained Ca, 31–37.2%; Fe, 0.2; I$_2$, 0.012; Cu, 0.025; Mn, 0.34; and Co, 0.002%.

TABLE 1. EFFECT OF FUROXONE SELF-FED TO SWINE FOR 81 DAYS ON RATE OF GAIN AND FEED EFFICIENCY

Treatment[a]	Average weight of swine		Average daily gain[c]	Feed per pound of gain
	Initial	Final		
	lb.	lb.	lb.	lb.
Negative	49.7	207.7	1.95	3.44
control	48.5	208.5	1.98	3.36
Furoxone				
5 gm.	49.2[b]	202.0	1.89	3.49
	50.6[b]	216.4	2.05	3.49
10 gm.	49.2	211.8	2.01	3.38
	48.5	211.8	2.02	3.42
15 gm.	49.0	209.3	1.98	3.44
	50.0	215.7	2.04	3.42
Chlortetracycline				
18 gm.	49.3	208.5	1.96	3.49
	49.7	205.8	1.93	3.46

[a] Two replicates of 6 animals per treatment.
[b] Autopsy determined no cause of death in either of the 5 gm. replicates.
[c] Analysis of variance indicated no significant difference between treatments at the 5% level (Snedecor, 1946).

TABLE 2. EFFECT OF FUROXONE FED AT VARIOUS LEVELS TO SWINE FOR 86 DAYS

Treatment[a]	Average weight of swine		Average daily gain[b]	Feed per pound of gain
	Initial	Final		
	lb.	lb.	lb.	lb.
Negative	44.7	185.0	1.63	3.67
control	44.7	185.7	1.64	3.34
Furoxone				
5 gm.	44.3	193.0	1.73	3.57
	45.2	182.5	1.60	3.41
7.5 gm.	44.5	187.5	1.66	3.62
	43.7	195.2	1.76	3.50
10 gm.	45.3	200.8	1.81	3.70
	42.3	187.7	1.69	3.57
50 gm.	42.8	191.3	1.73	3.46
	43.7	194.7	1.75	3.51
Chlortetracycline				
18 gm.	45.3	196.7	1.76	3.41
	43.5	198.2	1.80	3.53

[a] Two replicates of 6 animals per treatment.
[b] Analysis of variance indicated no significant difference between treatments at the 5% level (Snedecor, 1946).

required more feed per pound of gain, while the 5 and 50 gm. groups used less than the negative control. The average daily gains of the groups fed 10 and 50 gm. levels of Furoxone were 6.52 and 5.98%, respectively, greater than the negative control, while gains of the groups fed the 5 and 7.5 gm. levels of Furoxone were 1.62 and 4.4%, respectively, greater. The growth data tabulated in table 2 also failed to show statistically significant differences between the rations at the 5% level of probability.

In both studies, the Furoxone supplement did not elicit a significant growth response, as compared to the negative control alone. In the second trial, the results with Furoxone were inferior to those obtained with chlortetracycline, but the average daily gains were comparable, particularly at the 10 gm. level. The average feed efficiency for the positive control group, if both trials are to be considered, was slightly superior.

At all levels of Furoxone in the feed, the rations appeared to be palatable and non-toxic. There was no scouring in any group. Furoxone blended well in a batch of feed, as the analysis indicated less than a 0.005% difference between the top, middle, and bottom of the mixed ration.

The growth stimulation produced by certain drugs when used as feed additives in poultry and swine rations, is assumed to accrue as a result of the changed balance and environment of the intestinal microorganisms (Libby and Schaible, 1955; Owen and Elliott, 1954; Bowland, 1955).

Summary

Two trials were conducted with weanling Duroc pigs to compare 5, 7.5, 10, 15, and 50 gm. levels of Furoxone (furazolidone) per ton of feed to a recommended level of chlortetracycline and a non-medicinal negative control. The results showed:

1. Pigs fed Furoxone at several levels made slightly greater average daily gains than negative controls, but the differences failed to show statistical significance at the 5% level of probability.

2. In the first trial, pigs which received either 10 or 15 gm. of Furoxone in their ration, made slightly higher average daily gains than pigs receiving chlortetracycline, but in the second trial, the latter group made the highest average daily gain and required less feed for each pound of gain in weight than did pigs receiving several levels of Furoxone; however, none of the differences were significant at the 5% level.

3. The several levels of Furoxone fed in these trials failed to increase the feed efficiency consistently at all levels.

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COMPARISON OF CHROMOGEN METHOD WITH STANDARD DIGESTION TRIAL FOR DETERMINATION OF THE DIGESTABLE NUTRIENT CONTENT OF KIKUYU GRASS AND RAMIE FORAGES WITH SHEEP

ROBERT L. SQUIBB,[1] CARLOS RIVERA [2] AND ROBERTO JARQUIN [2]

THE importance of increasing the production of high protein forages in Central American areas has been stressed. Kikuyu grass *(Pennisetum clandestinum)* and ramie *(Boehmeria nivea)*, which can be grown throughout the American Tropics, have been shown to be of high nutritive value in animal diets (Squibb *et al.*, 1953 and Squibb *et al.*, 1954). Kikuyu grass grows at altitudes from 3,000 to 12,000 feet while ramie, which is an important fiber crop in Southern Florida, grows from sea level to 6,000 feet altitude. Little is known, however, of the digestible nutrient content of these forages especially when grown under Guatemalan conditions.

In these studies, digestion coefficients were determined for kikuyu grass and ramie forages using sheep in standard digestion trials, and the data were compared with those obtained simultaneously by the chromogen method of Reid *et al.* (1950).

Methods

Five yearling wethers, of approximately the same age and weight and of the breeding, Criollo 25% x Navajo 25% x Hampshire 50%, were selected for the digestion studies. These sheep were grazing either kikuyu grass or ramie at least one month before the corresponding trials. Standard fecal collection harnesses, modified to hold changeable plastic bags, were employed. During the trials the sheep were kept in small all-concrete pens.

In each trial there was a preliminary period of 7 days during which the animals received the test forage and water *ad libitum* under the actual conditions to be used in the experiment. Following this, each animal was fed a constant weight of the test forage over a 7-day period. Representative samples of each forage were obtained daily. The plastic bag inserts for collecting the feces were removed twice daily and the feces placed in sealed glass containers which were stored under refrigeration until analyzed. Forage and fecal samples were analyzed at the end of each digestion trial, and the calculation of the digestion coefficients was made by standard methods (Maynard, 1947). The same forage and fecal samples of these studies were also analyzed by the chromogen method of Reid *et al.* (1950), and these data used for comparison with the standard procedure.

The kikuyu grass was collected prior to maturity (less than 5 in. in

[1] Consultant in Animal Nutrition, Instituto de Nutricion de Centro America y Panama (INCAP), Guatemala, C. A. Present address, Dept. of Poultry Science, Rutgers University, New Brunswick, N. J.
[2] Servicio Cooperativo Interamericano de Agricultura (SCIDA), Guatemala, C. A. INCAP Scientific Publication I–89.

TABLE 1. CHEMICAL COMPOSITION OF KIKUYU GRASS AND RAMIE FORAGES (DRY BASIS)

Forage	Protein	Fat	NFE[a]	Crude fiber	Ash
	%	%	%	%	%
Kikuyu grass	15.18	4.31	28.57	39.30	12.64
Ramie, sample 1	20.57	2.74	44.12	16.77	15.80
Ramie, sample 2	19.48	2.53	23.38	37.99	16.62

[a] NFE=Nitrogen-free extract.

height) while the ramie samples were collected at the recommended height of 20 in. (Squibb *et al.*, 1954). Ramie sample 1 was collected in the rainy season and sample 2 during the dry season. Laboratory analysis of the forages and fecal samples were made by methods recommended by the A.O.A.C. (1950).

Results and Discussion

The chemical composition of the forages is presented in table 1. The data show these non-legume forages to be excellent sources of protein. The difference in fiber content of the two ramie forage samples was due

TABLE 2. COMPARISON OF THE DIGESTION COEFFICIENTS OF KIKUYU GRASS AND RAMIE FORAGES DETERMINED WITH SHEEP BY THE STANDARD DIGESTION TRIAL (STD) AND CHROMOGEN PROCEDURES (CHROM)

	Protein %		Fat %		NFE[a] %		Crude fiber %	
Sheep number	Chrom.	Std.	Chrom.	Std.	Chrom.	Std.	Chrom.	Std.
				Kikuyu grass forage				
1	64.7	62.1	57.0	53.9	69.6	67.9	67.7	65.4
2	67.6	64.3	63.8	60.2	76.9	74.5	65.8	62.4
3	60.2	60.6	57.7	58.1	74.0	74.3	64.9	65.4
4	59.5	59.9	58.1	58.6	77.0	77.3	58.0	58.5
5	61.7	60.9	55.0	54.2	72.5	71.9	62.8	62.1
Average	62.7	61.6	58.3	57.0	74.0	73.2	63.8	62.8
S. D.=	3.37	1.73	3.29	2.81	3.12	3.52	3.71	2.86
				Ramie forage, sample 1				
1	59.3	86.8	26.2	58.0	78.0	93.0	36.8	55.0
2	55.9	85.6	33.4	56.0	71.0	91.0	27.7	59.0
3	44.0	84.0	58.2	54.0	46.8	83.0	21.0	77.0
4	62.6	85.0	15.4	54.0	79.1	92.0	21.0	69.0
5	55.6	85.0	34.0	53.0	68.7	89.0	3.4	64.0
Average	55.5	85.3	33.4	55.0	68.7	89.6	22.0	64.8
S. D.=	7.02	1.02	15.74	2.00	13.03	3.97	12.24	8.61
				Ramie forage, sample 2				
1	68.8	85.0	2.6	54.0	39.8	88.0	74.5	71.0
2	68.6	83.0	9.6	49.0	71.8	79.0	62.9	84.0
4	69.5	81.0	18.3	48.0	36.0	89.0	82.6	59.0
5	69.7	83.0	9.6	49.0	77.4	73.0	52.4	87.0
Average	69.2	83.0	10.0	50.0	56.2	82.2	68.1	75.2
S. D.=	0.53	1.63	6.43	2.71	21.37	7.63	13.23	12.87

[a] NFE=Nitrogen-free extract.

to the age of the plants at the time of collection. Ramie grows to 20 in. in height more rapidly in the rainy season. The digestion coefficients, table 2, show that the nutrients of kikuyu grass are digested to about the same extent as those of Red top *(Agrostis alba)*. The digestion coefficients of ramie for protein, fat, nitrogen-free extract and crude fiber were superior to those of alfalfa and most clovers. The unusually high digestibility of the protein of this plant is further confirmation of its high nutritive value (Squibb *et al.*, 1953). Calculations of the digestible nutrient content of kikuyu and ramie forages are presented in table 3.

The results, table 2, show that there was excellent agreement between the data of the standard digestion trial and those obtained by the chromogen method for kikuyu grass. For the ramie forage samples, however, the two methods were not comparable. In the case of both ramie samples, the data

TABLE 3. DIGESTIBLE NUTRIENT COMPOSITION OF KIKUYU GRASS AND RAMIE FORAGES (DRY BASIS)

Forage	Protein	Fat	NFE[a]	Crude fiber	TDN
	%	%	%	%	%
Kikuyu grass	9.41	5.54	20.86	24.76	60.57
Ramie, sample 1	17.48	3.40	39.70	10.90	71.48
Ramie, sample 2	16.17	2.86	19.17	28.49	66.69

[a] NFE=Nitrogen-free extract.

obtained with the chromogen method were not only highly variable but yielded results which were significantly lower than those observed in the standard digestion trial. Apparently part of the chromogenic material in ramie is digestible. Since there is a possibility that forages, other than ramie, may also contain digestible chromogens, this method should not be used for determining the digestibility of little known or unknown tropical forages. The data do indicate that the chromogen method could be used for range studies with kikuyu grass.

Summary

Comparison of the chromogen method with standard digestion trials using sheep showed excellent agreement for kikuyu grass *(Pennisetum clandestinum)* and highly variable, low results for ramie *(Boehmeria nivea)*. These results preclude the use of the chromogen method for determination of digestion coefficients of little known or unknown tropical forages.

Both forages were similar to legumes in crude protein. The digestible nutrient content of kikuyu grass is similar to that of Red top *(Agrostis alba)*, while that of ramie is equal or superior to alfalfa and most clovers.

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CRYSTALLIZATION PATTERNS OF CERVICAL MUCUS AS RELATED TO ESTRUS IN BEEF CATTLE [1]

C. W. ALLISTON,[2] T. B. PATTERSON [3] AND L. C. ULBERG [2]

Mississippi Agricultural Experiment Station

A METHOD for predicting accurately time of estrus in large animals would be of considerable value in certain research projects. The crystallization patterns that form when cervical mucus is dried appears to have possibilities as a technique for prediction and/or detection of estrus. Bone (1954) used relatively elaborate methods for collecting the samples, and his primary interest was in utilizing the technique for early pregnancy diagnosis. This appears to be true of other reports dealing with the subject.

This preliminary study was undertaken to determine if the results obtained with a minimum of equipment would be consistent and could be used as an indication of approaching estrus.

Materials and Methods

A base heat date was established on 10 virgin beef heifers during October, 1956. A sample of cervical mucus was collected from each animal at approximate 12-hour intervals from the 16th day after estrus until such time as estrus and/or ovulation had occurred in each animal. The sample was collected by inserting a plastic inseminating tube into the cervix in the manner used in artificial insemination, and applying suction to the protruding end with an ordinary rubber bulb. The cervix was then manipulated around the tube, suction released and the tube withdrawn. The mucus was spread on a clean microscope slide, labeled for identification and dried at low temperature over an alcohol burner. A preliminary examination was made and the dried slide was stored for detailed study. Size of ovaries and ovarian structures was determined by rectal palpation immediately after each smear was made. Checks for heat were conducted within 30 minutes of the time of collection of cervical mucus samples, using a spayed heifer as the teaser animal.

Each author independently assigned each slide to one of the six arbitrary classes as described below and shown in figures 1 through 6. A stereomicroscope (9.9-60X) was used for examination. The final classification was determined by the median of the values assigned or by agreement of at least two authors as to its classification.

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[2] Present address: Department of Animal Industry, North Carolina State College, Raleigh.

[3] Present address: Department of Animal Husbandry, Alabama Polytechnic Institute, Auburn.

Results and Discussion

Since the length of the estrus cycle varied, the number of smears made from each animal varied. The results of smears taken during a period from 108 hours (4.5 days) before estrus until time of ovulation are shown in table 1 where 0 represents the time of first observed estrus. Heifer No. 9 did not exhibit estrus before ovulation. Although no reason can be given for this absence of estrus, its occurrence is not uncommon.

The values shown in the body of table 1 represent the following arbitrary categories on the basis of the appearance and extent of fernlike crystallization patterns:

(1) No pattern. (2) Patterns around bubbles only. (3) Typical patterns, but covering less than one half of the total slide area. (4) Typical patterns,

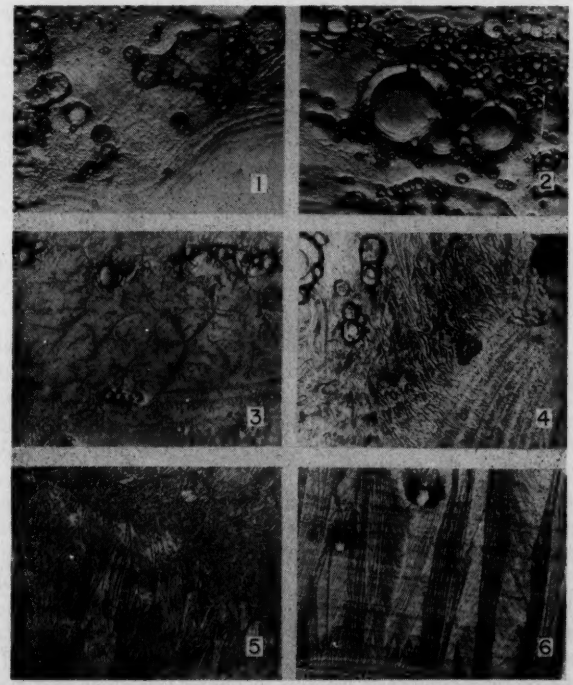

Figure 1. Six Arbitrary Classes of Crystallization Patterns of Cervical Mucus. (All magnifications 32x.) (1) No pattern. (2) Patterns around bubbles only. (3) Typical patterns, but covering less than one half of the total area. (4) Typical patterns, covering more than one half of the total area, but not complete. (5) Typical patterns over complete area, both short and long ferns present. (6) Typical patterns over complete area, only long ferns present.

covering more than one half of the total slide area, but not complete. (5) Typical patterns over complete area of slide, both short and long ferns present. (6) Typical patterns over complete slide area, only long ferns present.

The average first indication of the fernlike pattern occurred 84 hours prior to estrus (table 1). The average value increased gradually to a maximum of 4.4 at the time of estrus and began a decline prior to the average time of ovulation. Since estrus was not detected in heifer No. 9, the data from this animal are placed in table 1 on the basis of time of ovulation. These observations agree with those of Garm and Skjerven

TABLE 1. CRYSTALLIZATION PATTERNS OF CERVICAL MUCUS IN BEEF HEIFERS AS RELATED TO TIME OF ESTRUS

Hours prior to and after estrus	Coded slide value										Average value
	1	2	3	4	5	6	7	8	9[a]	10	
−108	..	1	1	1	1	1	1	..	..	..	1.0
−96	..	1	1	1	1	1	1	..	1	1	1.0
−84	..	1	1	2	1	3	1	..	1	1	1.4
−72	..	1	1	2	3	4	1	..	1	1	1.8
−60	..	3	3	1	3	3	1	..	3	2	2.4
−48	2	2	3	3	4	5	1	2	5	2	2.9
−36	2	3	4	3	5	4	3	3	2	4	3.3
−24	3	5	3	4	3	4	4	3	3	3	3.5
−12	3	..	5	5	5	5	5	5	2	3	4.2
0	5	5	5	5	5	5	5	3	3	3	4.4
12	3	6	1	5	3	5	5	4	1	..	3.7
24	5	4	5	4	1	5	5	1	3	5	3.3
36	3	..	1	4	..	..	..	2	2	..	2.4

[a] Estrus not detected, see text.

(1952) and Bone (1954) in that the fernlike patterns of crystallization were at a minimum during the luteal phase of the estrus cycle. Papanicolaou (1945, 1946) reported that in the human, fernlike appearance was at a maximum at the time of ovulation. In 1948 Ryberg confirmed these observations and determined that chlorides in the form of NaCl were responsible for the crystallization.

The regression of smear on follicle size appeared to be non-linear when the data were plotted. The data were therefore subjected to multiple regression analysis. Deviations from linear regression were highly significant (P 0.01). Therefore, extent of crystallization was not controlled by follicular size only.

At the time of estrus the cervical mucus was more abundant and less thick and adhesive than during the luteal phase of the cycle. This has been reported by a number of workers including Woodman and Hammond (1925), Hammond (1927) and Williams (1917). Campos da Pas (1951) has stated that the fernlike patterns of crystallization were an indication that the mucus secretion was favorable to the passage of sperm.

Vaginal mucus did not form crystallization patterns that were as consistent as cervical mucus. Bone (1954), also reported this observation.

In this study the crystallization patterns did not appear to be severely affected by the rate of drying. However, a more uniform slide was obtained when the heat was applied at a constant rate, but below the point at which the sample began to boil and/or bubble. Previous reports have been based on slides that were air dried.

Summary

This preliminary investigation indicates that the crystallization patterns of cervical mucus appears to be related to ovarian activity of the female. The patterns formed immediately before, during and after estrus are fernlike and more extensive than during the luteal phase.

The first indications of the fernlike patterns of crystallization of cervical mucus became evident about 3.5 days before estrus. These patterns reached a maximum in extent at the time of estrus and began a decline before ovulation occurred. Deviations from linear regression of smear on follicle size was highly significant. Vaginal mucus was less reliable than cervical mucus as an indicator of approaching estrus.

Although reasonable care must be exercised, extensive equipment and aseptic conditions are not essential for obtaining results that on the average appear to be satisfactory for predicting time of estrus within relatively narrow time limits.

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BEHAVIORAL, PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF GOATS TO TEMPERATURE, 0° TO 40° C.

ROBERT D. APPLEMAN [1] AND JAMES C. DELOUCHE [2]

Directorate of Medical Research, U. S. Army Chemical Corps [3, 4]

THE goat is used extensively as an experimental animal in Chemical Corps research. Some data are available on the physiological and biochemical characteristics of goats under normal environmental conditions (15° to 20° C.). Little is known, however, about the physiology and biochemistry of goats under adverse environmental conditions, particularly thermal stress. Further, the behavior of goats has not been charted in sufficient detail under any environmental condition. Knowledge of the behavioral, physiological, and biochemical responses in goats to temperature is basic to studies in which the reactions of goats to particular treatments are measured under other than "normal" environmental conditions.

Studies on the effect of various temperatures on the behavior and physiological reactions of the large (farm) animals have taken two forms. The earlier dealt with the effects of naturally occurring high temperatures in tropical or semi-tropical areas; the more recent work has been done in temperature-controlled laboratories. Techniques employed for the latter studies have been described by Brody (1948) and McDowell (1953). Animals (largely cattle) of various breeds are exposed to a wide range of controlled temperatures and their physiological reactions as reflected in milk yield, feeding habits, blood chemistry, cardiorespiratory activity and body temperature are compared. Brody (1948, 1956), Findley and Beakley (1954) and Lee and Phillips (1948) have presented reviews of results obtained.

Walker and Dziemian (1950) have investigated the physiological reactions of 24 goats to 23° C. environment. They observed a mean body temperature of 99.7° F., ranging from 98.6° to 102.2° F. Pulse rate averaged 89 beats per minute, varying from 75 to 110 per minute. Respiratory rates varied from 24 to 40 per minute. Blood hematocrits varied from 26.7 to 34.6%, with a mean of 30.2%. Plasma protein averaged 7.1 gm.%, ranging from 6.4 to 8.1 gm.%.

With the exceptions of eating, drinking and grazing activity, the behavioral responses of large animals to temperature has received little

[1] Present address: Extension Dairyman, University of Maryland, College Park, Maryland.
[2] Present address: Agronomy Department, Mississippi State College, State College, Mississippi.
[3] Army Chemical Center, Maryland.
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attention from investigators. Collias (1954, 1956) and Ross and Berg (1956) have reported on the effect of competition for food, water, or territory. They concluded that aggressive behavior starts as play-fighting among the kids and ultimately leads to the establishment of a rigid social hierarchy or dominance order.

Experimental Procedure

Four adult female goats, predominantly of the Nubian breed, characterized by having fine, short hair and being less hardy to cold than most breeds, were selected for this study. The animals received identical care with chamber temperature the only variable. They were offered legume hay and water *ad libitum*, while grain was fed daily at a rate of one pound per animal per day. The initial weight of the animals ranged from 35 to 43 kg.

The experiments were conducted in two temperature controlled chambers (one for chamber temperatures of 20° C. and above, the other for temperatures below 20° C.), the dimensions of which were approximately 10 x 25 feet.

Chamber temperature was varied from 20° to 30°, 35° and 40°, respectively, and from 20° to 10°, 5° to 0° C., respectively, over a six-month period. All periods were at least 12 days long to permit some acclimatization. The vapor pressure of the chambers was held constant at 12 mm. of Hg. in the warm environment while the relative humidity was maintained at 65% in the cool environment. The terms "chamber temperature", "ambient temperature","environmental temperature", or "temperature" employed in this report all refer to the dry-bulb temperature of the chamber air entering the outlet duct.

Quantitative data on behavioral characteristics were collected during 8 to 12 hours observation at each temperature. Most of the measurements involved time and were made with the aid of a stop watch. Water consumption was measured daily to the nearest 100 ml. by use of a graduated inverted water bottle feeding into a small open drinking container.

Physiological measurements (body temperature, pulse and respiration rates) were taken twice daily, morning and afternoon, each of the last three days of all environmental periods.

Body temperatures were taken with a calibrated rectal thermometer. Each reading was made at a constant depth of 3.5 inches and the insertion time was about three minutes.

Respiration rates were measured by counting flank movements with the aid of a stop watch when in cold environments. However, in warmer environments it was necessary to watch the movements of the *Dilatator naris lateralis* muscle of the nose or in some instances use a stethoscope on the dorsal portion of the back to detect clearly the respiration rate. The lowest rate recorded after the animal appeared calm was used.

Pulse rates were ascertained, by use of a stethoscope, through the ventral wall of the chest. Again, the lowest rates were recorded.

Blood samples were collected each morning of the last three days at each temperature. The samples were drawn into heparinized syringes from the jugular veins. Plasma was taken from blood centrifuged immediately after collection from the animal. There were no visible signs of hemolysis in any of the plasma specimens obtained.

The specifice gravities of blood and plasma were determined by the use of a series of bottles containing $CuSO_4$ solutions of known specific

TABLE 1. INFLUENCE OF TEMPERATURE ON TIME SPENT EATING, TOTAL DAILY WATER CONSUMPTION, TIME SPENT DRINKING AND DRINKING FREQUENCY

Observation	Goat	Ambient temperature in °C						
		0	5	10	20	30	35	40
Time spent eating (min./hr.)	A	20.0	24.1	25.1	20.1	17.5	12.5	0.9
	B	31.9	36.2	26.9	16.8	9.4	18.6	3.2
	C	21.6	16.9	17.6	15.5	7.3	11.1	0.4
	D	30.1	29.5	31.9	20.8	29.9	22.6	4.0
	Mean	25.9	26.7	25.4	19.3	16.0	16.2	2.1
Daily total water consumption (litters) [a]		9.2	9.6	9.2	11.5	18.0	26.4	16.0
Time spent drinking (sec./hr.)	A	10.3	10.0	10.5	16.5	24.5	23.8	15.4
	B	11.1	10.2	13.3	15.8	24.6	63.7	5.6
	C	9.8	9.0	16.9	17.1	15.6	39.8	7.6
	D	24.8	19.2	9.9	15.1	61.4	59.7	15.5
	Mean	14.0	12.1	12.6	16.1	31.5	46.8	11.0

[a] Combined consumption of the four goats.

gravities. Hematocrit levels were determined by using a graduated Winthrobe tube, centrifuging for exactly one hour at 3,000 r.p.m., and reading directly the cell pack percentage. Plasma chloride was determined by the micro-titration method. Plasma protein was found by determining the total nitrogen content by Key's micro-Kjeldahl method.

Results

Food and Water Consumption. The amount of time spent eating hay was the primary criterion of roughage consumption. The data (table 1) show that with hay supplied *ad libitum* time spent eating was considerably influenced by ambient temperature. Three levels of roughage intake corresponding to the low, medium and high temperature levels are evident. The animals spent on the average between 16 and 19 minutes per hour eating hay in the 20° to 35° C. temperature range. When the temperature was increased to 40° C., they practically stopped eating; two of the animals spent only 3 and 7 minutes, respectively, eating during 8 hours of

observation at this temperature. When the ambient temperature was lowered below 20° C., the animals spent about 25 minutes per hour eating hay; an increase of approximately 20% over the time spent at 20° C.

Very little foodstuffs were consumed at 40° C. Grain was nearly always consumed in a matter of minutes in the 0° to 35° C. environments. However, at 40° C. some grain was still left several hours after it was offered and frequently it was not entirely consumed in a 24-hour period.

Combined daily water consumption as well as the number of seconds spent drinking per hour by each goat during the 66 hours of observation showed a striking response to changes in ambient temperature (table 1).

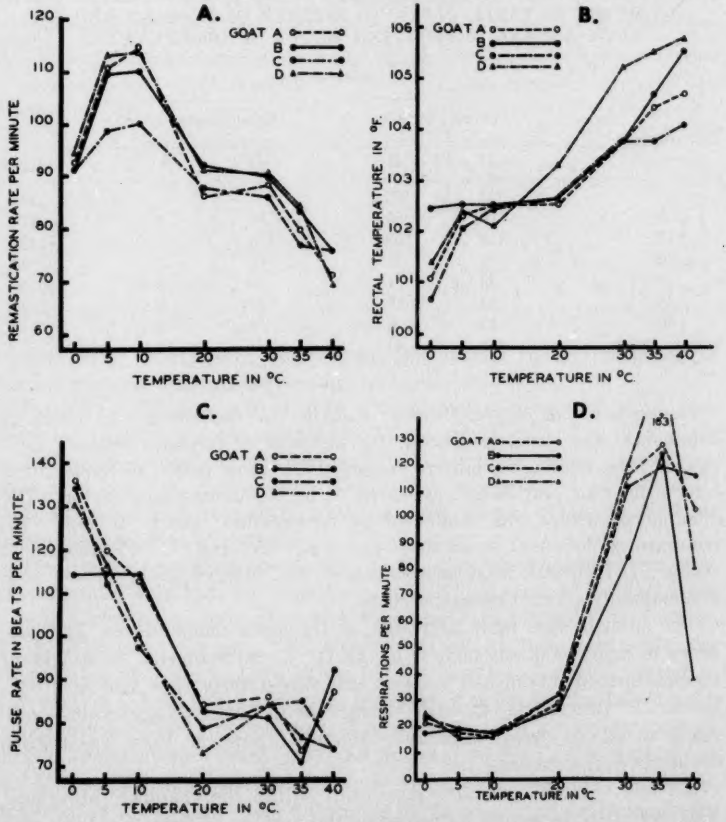

Figure 1. Effect of ambient temperature on the remastication rate, rectal temperature, pulse rate and respiration rate of goats. (Each point is an average of six measurements).

Rumination. Remastication rate was markedly affected by temperature. At 20° C. the mean rate was 90 per minute. When the temperature was lowered to 10° and 5° C., the mean rate increased to about 110 per minute. A further decrease in temperature to 0° C. depressed the remastication rate to one comparable to that at 20° C. The remastication rate at 30° C. was comparable to that at 20° C., however, the rate decreased at temperatures higher than 30° C., reaching a low of 73 per minute in the 40° C. environment. With the exception of goat "C", remastication rates at a given temperature were very stable (figure 1A).

TABLE 2. INFLUENCE OF TEMPERATURE ON AGGRESSIVENESS IN GOATS (NUMBERS IN TABLE REFER TO NUMBER OF WINS SCORED BY EACH ANIMAL DURING TEN HOURS OF OBSERVATION)

Environment °C	Number wins				
	Dominant pair		Subordinate pair		
	D	B	C	A	Total
0	93	47	1	1	142
5	64	50	10	0	124
10	128	100	3	1	232
20	26	17	0	1	44
30	24	7	0	0	31
35	21	15	12	0	48
40	13	0	1	0	14
Weight (kg.)	39	43	35	37	...

Dominance and Aggressiveness. Ranking of the animals in order of dominance was based largely on the outcome of combats between individuals. The dominance pattern remained the same under all temperature conditions. Size and weight appeared to be the determining factors. The dominance pattern was unaffected by temperature; goats "B" and "D" remained co-dominant at all times and goats "A" and "C" co-subordinate (table 2). However, total aggressiveness, i.e., number of aggressive acts, was markedly affected by temperature.

The animals were more aggressive at the lower temperatures. The incidence of aggression was highest in the 10° C. environment. At still lower temperatures it diminished but was still almost three time that at 20° C. In the 20° through 35° C. environments the incidence of aggression—with minor variations—was comparable. At 40° C., however, there was a sharp decline in aggressiveness.

Activity. Activity included movement within the chamber. Movement was arbitrarily measured by recording the number of times per hour each animal moved 10 feet or more from a stationary position and is reported in table 3.

Rectal Temperature. There was some evidence of a diurnal fluctuation in rectal temperature but it was not pronounced (figure 1B). Results from preliminary experiments indicated that the increase in body temperature associated with ambient temperatures of 30° C. or higher occurred during the initial 4 to 8 hours of exposure. After this initial rise body temperature leveled off and remained quite constant during the remaining period of exposure. In these preliminary experiments the rectal temperature of one animal rose to 107.5° F. within 6 hours after exposure to an environment of 50° C. Even though this body temperature was more than 5° F. above

TABLE 3. ACTIVITY RATINGS OF FOUR GOATS AT VARIOUS AMBIENT TEMPERATURES

Ambient temperature °C	Goat				
	Dominant		Subordinate		
	B	D	A	C	Mean
0	4.0	5.9	10.8	7.7	7.1
5	7.2	8.2	9.5	7.2	8.0
10	9.2	13.2	16.1	11.4	12.5
20	6.1	4.6	6.8	3.8	5.3
30	4.0	5.3	3.8	2.6	3.9
35	6.1	4.6	2.9	3.7	4.3
40	3.1	3.6	1.9	1.0	2.4

normal, after effects were not evident; the animal quickly returned to normal when the ambient temperature was lowered to 20° C.

Pulse Rate. There appeared to be a trend toward depressed pulse rates when the temperature was elevated to 35° C. (figure 1C). The pulse rates of two of the animals (goats "B" and "C") increased sharply upon subjection to a 40° C. environment. This might be attributed to a "stress" induced by the high temperature that was not present when the environmental level was 35° C. or below.

There was a prominent continuous rise in pulse rate when environmental temperatures were lowered from 20° C. to 10°, 5° and 0° C. The pulse rate in three of the four goats was about 65% higher at 0° C. than at 20° C. The curve for goat "B" indicates a rise in pulse rate when the temperature was lowered from 20° to 10° C.; however, further decreases in temperature did not effect additional increases of pulse rate for this animal.

Respiratory Rate. Respiratory rate rose rapidly with increasing environmental temperature (figure 1D). An increase in room temperature to 40° C. resulted in a sharp decrease in respiration rate from the peak exhibited at 35° C. Individual differences in response were not pronounced. The respiratory rate of goat "B" dropped much more sharply than the rates of other animals when the ambient temperature was increased from

35° to 40° C. This animal also maintained a stable respiratory rate in the lower environmental range (0° to 10° C.).

Blood Changes. There is some suggestion that hematocrit values were lower when climatic conditions became warmer (table 4). However, there is little indication that the whole blood:plasma specific gravity ratio is altered, which would be expected if hematocrit values were affected significantly by temperature.

Chloride values were lower on each extreme of the temperature gradiant than the values in the range of thermal neutrality (table 4). It appears

TABLE 4. EFFECT OF TEMPERATURE ON BLOOD HEMATOCRITS, WHOLE BLOOD SPECIFIC GRAVITY, PLASMA SPECIFIC GRAVITY, PLASMA CHLORIDES AND PLASMA PROTEINS. (VALUES ARE AVERAGE OF THE FOUR GOATS)

Ambient temperature	Hematocrit	Whole blood S.G.	Plasma S.G.	Plasma chlorides	Plasma protein
°C	%			mE/1	gm. %
0	33.4	1.0478	1.0258	93	...[a]
5	30.8	1.0438	1.0250	106	...[a]
10	29.5	1.0447	1.0244	106	...[a]
20	28.9	1.0444	1.0256	113	5.5
30	35.0	1.0456	1.0256	113	6.2
35	27.8	1.0446	1.0251	110	6.3
40	27.7	1.0456	1.0260	106	7.6
Mean	30.4	1.0452	1.0254	107	6.4

[a] No data collected.

that in the warm environments the decreased chloride values are due to a simple water dilution of the blood. This is supported somewhat by the fact that slightly lower hematocrit values were found when temperatures exceeded 30° C. This water dilution was, probably, a result of increased water consumption (for body-cooling purposes) with increasing ambient temperature. However, water consumption declined at 40° C. At 30° C. and above the drinking water was generally cooler than the environment.

Discussion

The responses to temperature of most of the characteristics considered here was compensatory. They reacted in a manner to allay environmental stress and particularly to maintain heat balance. They are not, however, necessarily independent of each other. The response pattern of several characteristics approximate each other so closely as to suggest more than a casual relationship between them.

The relationship between respiratory rate and water consumption is particularly suggestive. As respiratory rate increased and more water was vaporized, more water was consumed; when respiratory rates and va-

porization were minimized consumption decreased. In the 40° C. environment both respiratory rate and water consumption decreased considerably from the peak values obtained at 35° C.

The responses in food intake to temperature appear to be adaptive. Brobeck (1948) and Ragsdale *et al.* (1950) have pointed out that appetite behaves as if it were a thermo-regulatory mechanism. Since food consumption is associated with heat production, decreasing the temperature should bring about an increase in food consumption so as to increase heat production and keep the animals warm; food consumption should decrease with rising temperature so that heat production is curtailed and the animals kept cool. The results of these studies are in agreement with the above assumption.

Three other characteristics that showed an interesting parallelism of response are remastication rate, aggressiveness, and movement. Aggressiveness invariably involves movement. Aggressiveness and movement were greatest at 10° C. From this point they decreased as the temperature was raised or lowered. Remastication rate was similarly influenced by temperature indicating a possible relationship between activity (aggression, movement) and remastication rate. The nature of this relationship if it exists is not known, although the recent work of Larson's (1954) indicates that they might be under hypothalamic control and would, therefore, tend to respond similarly. The sensitivity of remastication rate to temperature changes makes it worthy of further consideration in studies on the environmental physiology of ruminants.

The tremendous increase in aggressiveness at the lower temperatures appeared to be related to an increase in general "activity" of the animals. Scott (1945) has pointed out that sheep in periods of cool or cold weather invariably bring on unusual amounts of activity. Some of this increased activity may be a result of an attempt by the animals to keep warm by increasing heat production. This interpretation is probably applicable to the present observed increase in aggressiveness at the lower temperature. Aggressiveness is a form of activity which increases heat production. There is, however, another more direct and perhaps equally substantial interpretation of the rise in aggressiveness under cool environments. The animals spent considerably more time eating hay in the cool environments. Since the amount of feeding space was the same throughout the studies, the increase in time spent eating at the lower temperatures might have resulted in greater competition for food and feeding space. The fact that most aggressive actions regardless of temperature took place around the hayrack or grain trough support this interpretation.

With the exception of the 40° C. environment high temperatures elicited responses that lowered heat production and increased heat loss. In the 30° and 35° C. environments, heat production was decreased by lowered food consumption and decreased general activity while heat loss was increased principally through increased respiratory activity. At 40° C., however, the animals were apparently beyond their heat tolerance limits. Deterio-

rative processes assumed dominance and the physiological well being of the animals steadily declined. They practically stopped eating, aggressive activity was subnormal, and the respiratory rate was characterized by a dramatic decline.

Exposure to cold as low as freezing had little detrimental effect on the animals. Compensatory reactions were initiated to maintain heat balance, but apparently the heat-regulatory mechanisms were not heavily taxed. Heat production was raised through increased food consumption and activity. Some shivering occurred. Heat loss was minimized by low respiratory rates, reduced water consumption, and piloerection. Pulse rate steadily increased as the temperature was lowered below 20° C. This reaction was probably associated with increased heat production (Kibler and Brody, 1949). At 0° C., the rectal temperature of 3 of the 4 animals dropped about 1° F. This would seem to indicate that the limit of cold tolerance was approached. Further studies should be undertaken employing temperatures below freezing to determine the cold tolerance limits of Nubian goats.

Summary and Conclusions

Decreasing the environmental temperatures from 20° to 0° C. resulted in: (a) an increase in time spent consuming roughage, (b) a slight decrease in water consumption, drinking frequency and time spent drinking, (c) a considerable increase in remastication rate except in the 0° C. environment when the rate dropped to a level comparable to that of 20° C., (d) an increase in aggressiveness, time spent standing and movement, (e) a 1° F. drop in rectal temperature, a slight depression in respiration rate and a dramatic rise in pulse rate, and (f) no appreciable change in hematocrit, or specific gravity of plasma and whole blood; but a decrease in plasma chloride.

Increasing the temperature from 20° to 40° C. resulted in: (a) a decrease in time spent consuming roughage, (b) an increase in water consumption, drinking frequency and time spent drinking except for a pronounced decrease in each at 40° C., (c) a decrease in remastication rate, aggressiveness, time spent standing and movement, (d) an increase in rectal temperature, little change in the pulse rate pattern and a tremendous increase in respiration rate except for a sharp decline at 40° C., (e) no apparent change in either plasma or whole blood specific gravity, but a suggestion of lowered hematocrit values, and (f) a decrease in plasma chlorides and an increase in plasma protein.

It was found that the limit of heat tolerance for the goats lay between 35° and 40° C. Failure of the heat regulatory system occurred at 40° C. The cold tolerance limits were not approached even when the temperature of the environment was lowered to 0° C. for 288 hours duration.

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A STUDY OF THE REPRODUCTIVE PERFORMANCE OF A PUREBRED HEREFORD HERD [1]

C. E. LINDLEY,[2] G. T. EASLEY,[3] J. A. WHATLEY, JR.[4] AND DOYLE CHAMBERS [4]

Oklahoma Agricultural Experiment Station

IN general the reproductive efficiency of beef cattle has been low (Phillips, 1939; Ensminger *et al.*, 1955; and Johnson, 1930). Phillips (1939) pointed out that reproduction in cattle can be affected by many things. Unfortunately only a small part of the variation in reproductive performance can be accounted for by genic variance, as indicated by low repeatability and heritability estimates (Brown *et al.*, 1954; Warnick, 1955; Carman, 1955; Legates, 1954; Olds and Seath, 1950; Dunbar and Henderson, 1953; and Pou *et al.*, 1953). Certain environmental factors have been shown to materially affect reproductive performance. Age of dam (Lasley and Bogart, 1943; Davis, 1951); age of bull (Erb *et al.*, 1940; Bowling *et al.*, 1940; and Bishop *et al.*, 1954); season (Morgan and Davis, 1932; Clapp, 1956; Mercier and Salisbury, 1956; and Phillips *et al.*, 1943); year (Baker and Quesenberry, 1944, and Warnick, 1955); and length of sexual rest (Trimberger, 1954; and Lasley and Bogart, 1943) have all been observed to be important environmental factors affecting reproductive performance.

Most of the information available on reproductive performance concerns dairy cattle. The purpose of this study was to investigate further the reproductive performance of beef cattle.

Materials and Methods

The data for this study were taken from the breeding records of the Turner Hereford Ranch, Sulphur, Oklahoma for the period from 1935 through 1952. There were 848 cows with 3,606 gestations included in the study. A total of 123 bulls sired calves during this period. The herd was managed according to good practices and was relatively free of disease.

The cows were bred for year-around calving except for the last few years when summer calving was avoided. Since only a small number of gestations were involved during this period, not breeding cattle during the fall for this short period was not considered to be important in influencing the overall performance. The cows were hand mated, with the exception

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[2] Head, Animal Husbandry Department, Mississippi State College.

[3] Veterinarian, Turner Ranch, Sulphur, Oklahoma.

[4] Animal Husbandry Department, Oklahoma State University, Stillwater.

of a few pasture matings, from 1935 to 1946. After 1946, the breeding was entirely artificial. The breeding data were recorded on a card for each cow.

To remove seasonal and yearly variation, the data were adjusted to the average for the summer season of 1939. This season and year were selected because fewer adjustments of the data would be required.

The date of breeding was used as the base for determining age of cow,

TABLE 1. SEASONAL AVERAGES OF REPRODUCTIVE PERFORMANCE

Season of breeding	Successful services	Intervals calving to conception	Gestation length	Calving intervals	Intervals calving to first breeding	Intervals first breeding to conception
		days	days	days	days	days
Spring						
Number	619	504	641	498	494	494
Average	1.8 S/C[a]	112.3	286.0	399.5	72.6	38.7
Summer						
Number	1177	1174	1392	1165	1137	1139
Average	1.6 S/C	105.4	285.8	391.9	79.4	23.5
Fall						
Number	646	580	719	569	566	566
Average	1.7 S/C	105.7	285.5	390.5	74.4	30.1
Winter						
Number	807	665	854	655	655	655
Average	1.8 S/C	112.0	285.5	397.4	72.0	39.1
Total						
Number	3249	2923	3606	2887	2852	2854
Average	1.7 S/C	108.2	285.7	394.2	75.5	31.0

[a] S/C=services per conception.

season of breeding, and year. The data were punched on IBM cards for ease of tabulation. The statistical methods used were those described by Snedecor (1946). The repeatability estimates were computed from components of variance as intra-class correlations. The heritability estimates were computed as four times the half-sib correlation, twice the regression of daughter records on dam records, and twice the correlation between the records of daughter and dam. These regressions and correlations were determined from daughter and dam averages.

Results and Discussion

Yearly and Seasonal Effects. While the reproductive efficiency of the herd declined gradually during the period covered by this study, analysis of variance indicated no significant variation due to year except for number of services per conception. The average number of services per conception increased from 1.4 in 1935 to 2.0 in 1951.

Part of this increase may be accounted for by the change from natural service to artificial insemination. Under natural service it was often necessary to conserve the bull. This was not true with artificial insemination. The difficulty of checking heat resulted in cows being bred whenever they showed signs of heat. This resulted in a lower conception rate. The average reproductive performance for the 16-year period is given at the bottom of table 1.

TABLE 2. CORRELATIONS AND REGRESSIONS OF REPRODUCTIVE PERFORMANCE ON AGE OF COW IN HALF YEARS

Measure	r	95% Confidence limits	b	95% Confidence limits
Number of services per conception	0.64**	0.32 0.83	0.02**	—0.10 0.14
Gestation length	0.64**	0.35 0.82	0.12**	0.06 0.18
Calving interval	0.47*	0.08 0.74	1.59*	0.22 2.96
Interval calving to first breeding	0.46*	0.11 0.70	0.66*	0.10 1.92
Interval first breeding to conception	0.43*	0.07 0.70	0.99*	0.06 1.92
Interval calving to conception	0.64**	0.37 0.82	2.05**	0.97 3.13

* ($P<.05$).
** ($P<.01$).

The seasonal averages for the reproductive performance are shown in table 1. In general there was a tendency for reproductive performance to be improved in those cows that were bred in summer and fall. Approximately 0.15 less services per conception were required in summer and fall than in spring and winter. The interval from first breeding to conception averaged 13.2 days less in summer and fall than in the other two seasons. The calving intervals and intervals from calving to conception averaged 7.0 and 6.6 days less, respectively, for cows bred in summer and fall. The interval from calving to first breeding increased 5.4 days during summer and fall. This may account for some of the improvement in reproductive performance in summer and fall. Trimberger (1954), Van Demark and Salisbury (1950), and Lasley and Bogart (1943) found that an increase in this interval improved breeding efficiency. The improved nutrition during the pasture season may explain this improved reproductive performance, or cattle may tend to be seasonal breeders.

Effect of Age of Cow. The correlations between the age of the cow and reproductive performance and the regressions of reproductive performance on age of cow are shown in table 2. All these correlations and regressions

are positive and significant, indicating that as cows become older, reproductive performance declines. However, when tested for deviations from linearity, all the regressions, except that for gestation length on age of cow, showed significant deviations, indicating that the relationship was curvilinear. The multiple-range test indicated that young cows and old cows were significantly different from cows of intermediate ages in length of calving interval and that old cows had significantly longer intervals from calving to first breeding and from first breeding to conception. No differences were indicated by the multiple range test between ages in number of services per conception. However, older cows again tended to require

TABLE 3. REPEATABILITIES AND HERITABILITIES OF REPRODUCTIVE PERFORMANCE

	Repeatability	Heritability		
		p	r	b
Number of services per conception	0.07	0.05	−0.18	−0.24(356) [a]
Gestation length	0.15	−0.05	0.04	0.24(364)
Calving interval	0.06	0.07	0.14	0.14(309)
Interval calving to first breeding	0.03	0.02	0.22	0.30(308)
Interval first breeding to conception	0.06	0.04	0.01	0.01(308)
Interval calving to conception	0.07	0.04	0.12	0.14(313)

p Estimated from paternal half-sib correlation.
r Estimated from correlation of daughter-dam records.
b Estimated from regression of daughters' records on dams' records.
[a] Numbers in parentheses indicate the number of daughter-dam pairs.

more services per conception. Analysis of variance indicated significant differences among ages in all those traits.

Repeatability and Heritability of Reproductive Performance. The repeatability and heritability estimates of reproductive performance are given in table 3. The repeatability and paternal half-sib heritability estimates were determined by the intra-class correlations from components of variance. All estimates were generally low. Repeatability estimates as low as these indicate that culling cows on the basis of early records for reproductive performance would be of little value. Future performance cannot be predicted accurately from early records when the repeatability estimates are as low as these. The low heritability estimates indicate that there is very little genetic variability in the traits measured. The fact that some of the estimates were negative and others positive indicates that the true estimates are near zero. The accuracy of predicting the performance of daughters from the records of the dams is very low when heritability estimates are as low as these. Progress by selection for these traits would be exceedingly slow. In a selection program, effort could well be expended on other more highly heritable traits. These estimates in general agree with those found in the literature (Carman, 1955; Legates,

1954; Olds and Seath, 1953; Dunbar and Henderson 1953; and Pou *et al.*, 1953).

The estimates from the correlation between daughter and dam records and the regression of daughter records on dam records were generally larger than those determined by paternal half-sib correlations. Lush (1948) stated that environmental variations may be correlated between individual parent and offspring and that some of the epistatic deviations will be correlated between parent and offspring. This may account for the larger estimates by the daughter-dam correlations. Sampling errors might cause the variation in heritability estimates by the different methods. This could also be due to the use of averages in computing these estimates.

TABLE 4. AVERAGE NUMBER OF SERVICES PER CONCEPTION FOR VARIOUS LENGTH INTERVALS FROM CALVING TO FIRST BREEDING

Interval from calving to first breeding	Number of services per conception	Number of cows
days		
27–50	1.94	32
51–60	1.76	52
61–90	1.38	426
Over 90	1.33	225

The heritability estimates determined by the regression of daughters' records on dams' records were generally larger than those determind from the correlation of these records. Lush (1948) stated that this is to be expected when the parents are selected.

Effect of Length of Interval from Calving to First Breeding on Number of Services per Conception. In this study the regression of the number of services per conception on the length of interval from calving to first breeding was —0.10 ($P<.01$), indicating that cows bred at shorter intervals after calving required more services per conception than those bred after longer intervals. The number of services per conception for different length intervals from calving to first breeding is given in table 4. Cows bred from 27 to 50 days after calving required 1.94 services per conception; those bred 51 to 60 days required 1.76; those bred 61 to 90 days required 1.38; and those bred over 90 days required 1.33. It might be desirable to wait until 50 or 60 days post partum before rebreeding. Data reported by Trimberger (1954); Van Demark and Salisbury (1950); Shannon *et al.* (1952), and Lasley and Bogart (1943) indicate that excessively short intervals from calving to first breeding are not desirable.

Additional Observations. The reproductive life of a cow is economically important to the beef producer. A long reproductive life for cows enables the breeder to make fewer replacements and thus have more young breeding animals to market and permits greater selection pressure. The average

age of the 530 cows removed from the herd for all reasons during this study was 7.8 years. These cows had an average productive life of approximately five years. Those cows that were removed from the herd when they failed to reproduce regularly or failed to produce desirable calves had an average age of 9.2 years. Cows culled on pedigree were included in this group. All cows that had reached this age at the end of this study had produced an average of 5.6 calves.

There were 27 cows during the 16 year period that failed to produce a calf from a average of 8.8 services. These were classified as barren cows and sold. This group included 3.2% of the cows.

Summary

The reproductive performance of a purebred Hereford herd was studied for the period from 1935 through 1952. The reproductive performance of this herd gradually declined during this period. The average reproductive performance for this period was: number of services per conception, 1.7; interval from calving to conception, 108.2 days; gestation length, 285.7 days; calving interval, 394.2 days; interval from calving to first breeding, 75.5 days; and interval from first breeding to conception, 31.0 days. In general, the reproductive performance was higher in summer and fall than in spring and winter.

Cows more than 10 years of age declined rapidly in performance. All correlations and regressions of performance on age of cow were positive and significant. The rapid decline at the older ages caused the regression to be curvilinear.

During the period of this study 530 cows were taken from the herd at an average age of 7.8 years. Cows that were sent to market at the end of their productive life had an average age of 9.2 years. Thus they had a productive life of 7.5 years.

Heritability estimates of reproductive performance were determined by the paternal half-sib intra-class correlation, the correlation between daughter and dam records, and the regression of daughters' records on dams' records. The repeatability estimates were determined by intra-class correlations. The estimates were generally low. Most heritability estimates were near zero. Repeatability estimates varied from 0.03 to 0.15.

Cows bred more than 60 days post partum required fewer services per conception than those bred prior to 60 days post partum.

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EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON GROWTH AND UPON CALCIUM AND PHOSPHORUS METABOLISM IN LAMBS [1]

JOSEPH D. SHRODER [2] AND SAM L. HANSARD [3]

University of Tennessee—Atomic Energy Commission Agricultural Research Program

THE favorable effects of stilbestrol upon rate of gain and feed efficiency have been widely accepted but few attempts have been made to determine the mechanism of action of this material. Several investigators (O'Mary *et al.*, 1952; Dinusson *et al.*, 1950; Perry *et al.*, 1955) have reported increased daily feed consumption by ruminants receiving stilbestrol. Whitehair *et al.* (1953) reported that implanting stilbestrol increased calcium, phosphorus, and nitrogen retention in lambs, results confirmed by Bell *et al.* (1955) with orally administered stilbestrol. Jordan (1953) in experiments with lambs and Clegg (1952) and Clegg and Cole (1954) with steers observed a marked increase in nitrogen retention as a result of stilbestrol administration. O'Mary *et al.* (1952) observed a greater amount of total bone in lambs receiving stilbestrol implants, but Wilkinson *et al.* (1955) reported that stilbestrol had little influence on percentage of bone ash in growing lambs. This has been confirmed by Clegg and Carroll (1956) with steers. These findings would indicate a possible growth stimulation associated with stilbestrol treatment. This report is concerned with the effect of dietary stilbestrol upon skeletal growth, and upon the absorption, excretion, and physiological behavior of calcium and phosphorus in lambs.

Experimental

Twenty-eight crossbred wether lambs averaging approximately 75 lb. in weight were used in this experiment. They were maintained on a ration of orchard grass hay and a concentrate mixture (75% yellow corn, 15% alfalfa meal, 10% soybean oil meal, and 10 gm. of irradiated yeast per hundred weight) previous to and during the experimental period. Eighteen of the lambs were divided at random into two groups of 9 animals each.

[1] A portion of a dissertation presented by the senior author to the Graduate School, Oklahoma State University, in partial fulfillment of requirements for the degree of Doctor of Philosophy.

[2] Formerly Graduate Fellow, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee. Present address: Department of Animal Husbandry, Iowa State College.

[3] Present address: Department of Animal Industry, Louisiana State University. This manuscript is published with the approval of the Director of the University of Tennessee Agricultural Experiment Station, Knoxville, Tennessee. The radioactive materials used in this work were obtained from the Oak Ridge National Laboratory on allocation from the United States Atomic Energy Commission. This work was completed under Contract No. AT-40-1-GEN-242 between the University of Tennessee, College of Agriculture and the Atomic Energy Commission.

One group received the basal ration and the other group received the basal ration plus 2 mg. of stilbestrol per head daily. All animals were placed in metabolism units initially and at 3-week intervals during the 60-day feeding trial for 7-day chemical and radio-chemical blood and balance studies using procedures described by Hansard *et al.* (1954b). In brief, following the preliminary period in metabolism units, the animals were given a single oral or intravenous dose of calcium-45 and phosphorus-32, concurrently. During the collection period representative samples of urine and feces were taken from each daily collection and placed in separate containers for individual analysis. Blood samples were taken periodically and, following the completion of the balance study, the animals were removed to their respective lots in preparation for the next balance period.

Aliquots of feed, feces, and urine were ashed in a muffle furnace, dissolved in 6 N HCl, and diluted to a known volume with re-distilled water. Phosphorus was determined on blood plasma and on aliquots of the dissolved ash by the method of Fiske and Subbarow (1925). Urinary and plasma phosphorus-32 was assayed in 10 ml. of fresh urine or 5 ml. of plasma; fecal radiophosphorus was determined by counting 10 ml. aliquots of the dissolved ash by conventional procedures.

Analyses of calcium in plasma, feeds, and urine were accomplished titrimetrically by the method of Clark and Collip (1925). Fecal calcium was determined gravimetrically by weighing the oxalate precipitate from an aliquot of dissolved ash. Radioassay for calcium-45 in plasma, feces, and urine was performed by the methods described by Comar *et al.* (1951).

Endogenous calcium and phosphorus losses were measured by the comparative balance procedure (Hansard, 1957) and true digestibility was calculated by the conventional formula.

The remaining 10 lambs were placed in drylot and when they were consuming enough feed to permit rapid growth, they were divided at random into two groups of five lambs each and individually dosed intramuscularly with a single tracer dose of calcium-45 to mark the bones for autoradiographic studies. One group continued to receive the basal ration while the remaining lambs received the basal ration to which was added 2 mg. of stilbestrol per pound of feed. Throughout the experimental period the lambs consumed approximately 2 lb. of concentrate and 2 lb. of hay per head daily. They were weighed without shrink at the beginning and at 2-week intervals during the experimental period. At the end of a 75-day feeding period the lambs were weighed and sacrificed and the right femur bone of each removed for preparation of radioautographs by the method of Lotz *et al.* (1952).

Results and Discussion

Effects upon Growth. Response of lambs to stilbestrol feeding in this study is shown in table 1. The difference observed in average daily gains

between the control and treated animals was not significant at the 5% level.

Hansard (1957) and Hansard *et al.* (1954a) demonstrated that when farm animals are dosed with radiocalcium the retained isotope is rapidly deposited in the sub-epiphyseal area of the bone directly beneath the epiphyseal plate and remains there until removed by growth and/or exchange. New bone formed, having a lower concentration of radioactivity, appears lighter in tone on the autoradiograph, thereby providing a visual indication of bone growth and making possible quantitative measurements of animal response to treatment. This is demonstrated in the saggital sections of femurs from control and treated lambs, examples of which appear in

TABLE 1. EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON WEIGHT GAINS AND BONE GROWTH IN LAMBS

Comparisons	Basal	Stilbestrol
Number of animals	5	5
Weight gain, lb.[a]	30.4	34.8
Average daily gain, lb.	0.41	0.47
Bone growth of femur, cm.		
Proximal end	0.28	0.32
Distal end	0.53	0.63
Bone growth of femur, cm./lb. gain $\times 10^3$		
Proximal end	9.4	9.1
Distal end	17.9	18.2

[a] Total gain over a 75-day feeding period.

figure 1. Bone laydown and growth during the 75-day feeding period is represented by the gray area in the epiphyseal region shown on the autoradiograph. Bone growth measurements are summarized in table 1.

In this study bone growth measurements were made with precision calipers on 4 to 6 exposures of sections from each lamb femur and the results averaged to give the centimeters of bone growth reported. It is of interest to note that the same percentage difference in growth occurred at the proximal and distal ends of the bones of each group. It is pointed out that the bone growth measurements at the proximal end of the femur were not significantly different for the control and treated lambs, however, at the distal end, bones from the treated animals increased 0.63 cm. in length as compared to 0.53 cm. for the controls; measurements highly significantly in favor of the stilbestrol-fed lambs ($P<.01$). When bone growth from the control and treated groups was compared on the basis of centimeters per unit gain in weight, however, there were no differences between lots.

These data and other findings indicating a significant increase in pituitary growth hormone (Shroder and Hansard, 1957) support the idea that the primary effect of stilbestrol in ruminants is that of growth stimulation. The amount of total bone in the treated lambs was increased as reported by O'Mary *et al.* (1952). However, when expressed as a percentage of body weight there was no increase in bone as compared to other tissue.

The results of Wilkinson *et al.* (1955) with lambs and of Clegg and Carroll (1956) with cattle support these observations.

Effects upon Calcium and Phosphorus Retention. Summaries of the calcium and phosphorus balance data are presented in table 2. Average calcium retention for lambs receiving the basal ration was 0.09 gm. per head daily, while the stilbestrol-fed lambs averaged 0.57 gm. per head daily, a highly significant difference ($P<.01$). For the control lambs, average phosphorus retention was —0.25 gm. per head daily compared to an average retention of 0.05 gm. per head daily by the stilbestrol-fed lambs, also a highly significant difference ($P<.01$). Apparent digestibility of both calcium and phosphorus was in favor of the stilbestrol-fed lambs ($P<.01$). Thus for the control and stilbestrol-fed animals, apparent digestibility of calcium was 4.3 and 15.2%, respectively, and of phosphorus, —5.7 and 4.8%, respectively. These calcium and phosphorus balance data are in agreement with results reported by Whitehair *et al.* (1953) using stilbestrol implants and by Bell *et al.* (1955) with orally administered stilbestrol.

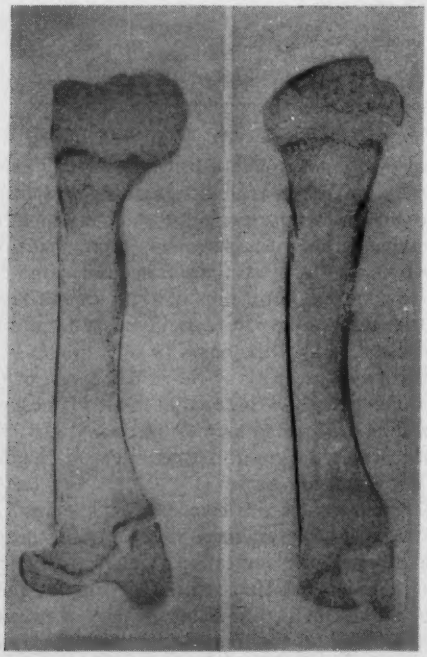

Figure 1. Autoradiographs of sagittal sections of femur bones from control (left) and stilbestrol-fed (right) wethers demonstrating the growth effects.

Effects upon Blood Radioactivity Concentration. Radioactivity in the total blood as a function of time after administration of a single intravenous or oral dose of calcium-45 or phosphorus-32 was calculated from the blood concentration and blood volume values as described by Hansard *et al.* (1953). It is graphically illustrated in figure 2.

Since the blood represents the central metabolic pool through which all ions must pass in the process of absorption from the gastrointestinal tract,

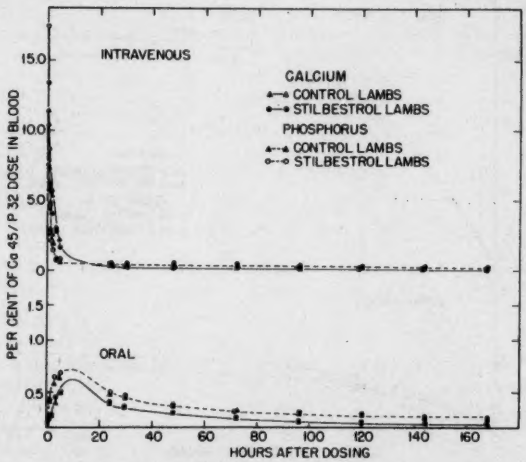

Figure 2. Calcium-45 and Phosphorus-32 in the total blood of control and stilbestrol-fed wether lambs as a function of time following oral or intravenous administration.

deposition in the bone and soft tissues, excretion, and transfer from one tissue to another, the blood levels of calcium-45 and phosphorus-32 at a given time theoretically would be useful in interpreting the overall behavior of calcium and phosphorus in the animal body. Following intravenous administration of the radioactivity, it is noted that disappearance from the blood followed a characteristic curve and that stilbestrol had no significant influence either upon the blood levels of the isotopes or upon the rate of disappearance.

Following oral administration, the blood curves reflect the difference between two opposing processes; absorption from the tract and removal from the blood. After about 100 hours, however, the level of radioisotopes in the total blood is governed primarily by the exchange between the bones and the plasma (Hansard *et al.*, 1954a) and should reflect any marked difference due to treatment. It is apparent, from inspection of the curves, that dietary stilbestrol was without effect in altering the rate of movement

of calcium or phosphorus between the gastrointestinal tract, blood, bones and tissues.

Effects upon Fecal Excretion. The rate of fecal excretion of calcium-45 and phosphorus-32 is graphically illustrated in figure 3. The accumulative curves representing the excretion of the orally administered radioisotopes exhibit two distinct slopes; the initial, relatively steep, exogenous slope, and

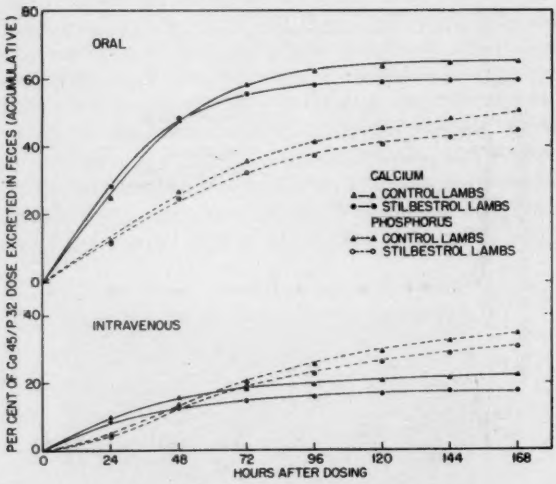

Figure 3. Accumulative fecal excretion of orally and intravenously administered calcium-45 and phosphorus-32 by control and stilbestrol-fed wether lambs.

the endogenous slope characterized by a lower rate of excretion. For calcium, the exogenous slope representing primarily unabsorbed calcium-45 excretion, was essentially the same for both the control and treated lambs, indicating that calcium absorption *per se* was apparently unaffected by stilbestrol feeding. The divergence of the phosphorus excretion curves throughout the exogenous phase, however, reflects an increased absorption of phosphorus due to stilbestrol feeding. During the endogenous phase, the curves representing the appearance in the feces of calcium-45 and phosphorus-32 derived from the body indicate that stilbestrol reduced the amount of both calcium and phosphorus excreted from body stores. These results are in agreement with the calculated values from the balance studies (table 2) to be discussed later.

Following intravenous administration of a single dose of radioactivity, the accumulative excretion curve represents the net fecal excretion after all physiological processes have had their effects and is indicative of the

endogenous fecal fraction. By the definition to be used hereafter the term "fecal endogenous" will apply to any calcium or phosphorus appearing in the feces which has passed through the plasma pool. From the intravenous excretion curves, a marked reduction in fecal endogenous calcium-45 and phosphorus-32 was noted in the stilbestrol-fed animals. In all animals,

TABLE 2. EFFECTS OF ORALLY ADMINISTERED STILBESTROL UPON CALCIUM AND PHOSPHORUS ABSORPTION AND EXCRETION IN LAMBS

Items	Calcium		Phosphorus	
	Basal	Stilbestrol	Basal	Stilbestrol
Number of animals	9	9	9	9
Number of trials	3	3	3	3
Average weight of animals kg.	38.3	39.9	38.0	39.5
Chemical balance data, daily gm.				
Intake	4.10	4.16	2.82	2.94
Feces	3.93	3.52	2.98	2.79
Urine	0.08	0.07	0.09	0.10
Net retention	+.09	+.57	−.25	+.05
Radiochemical data[a]				
Percent Ca-45 in feces:				
Intravenously administered	22.1	18.0	35.5	31.2
Orally administered	65.5	60.0	51.1	45.1
Fecal endogenous loss[b]	43.9	34.7	61.9	57.7
Apparent digestibility, %	4.3	15.2	−5.7	4.8
True digestibility,[c] %	45.1	48.6	77.7	82.7

[a] In each trial two-thirds of the animals were dosed orally and one-third were dosed intravenously with tracer levels of Calcium-45 and Phosphorus-32; total Ca and P values are averages for all animals.
[b] Expressed as mg. per kg. of body weight per day.
[c] Calculated as $100 - \frac{(\text{Fecal mineral} - \text{Fecal endogenous mineral})\ 100}{\text{Mineral intake}}$

the urinary excretion of the radioisotopes was negligible and is not considered further.

A summary of the chemical and radiochemical calcium and phosphorus balance data, indicating the effects of dietary stilbestrol upon absorption and excretion, is presented in table 2. It must be pointed out that the behavior of a single dose of radioactivity reflects the movement of the current dietary intake only and is influenced by the nutritional history of the animal and current mineral status (Hansard *et al.*, 1951).

Following intravenous administration of the radioactivity, 22.1% of the calcium-45 was excreted in the feces of the control lambs as compared to 18.0% for those animals receiving stilbestrol, a difference significant at less than the .09 level. Of the injected radiophosphorus, 35.5% was excreted by the control lambs while those receiving stilbestrol excreted 31.2%, a highly significant difference ($P < .01$). These data indicate that

the treated lambs utilized the absorbed calcium-45 and phosphorus-32 more efficiently than did their controls.

Following oral administration of radiocalcium, the control lambs excreted approximately 65.5% of the dose in the feces as compared to 60.0% by those receiving stilbestrol, a difference significant at the .10 level; whereas the control lambs excreted 51.4% of the orally administered radiophosphorus contrasted with 45.1% excreted by the treated animals ($P<.05$). Whether these differences were due to increased reabsorption within the gastrointestinal tract or to decreased re-excretion of the absorbed calcium or phosphorus in the treated animals will require further study.

Effects upon Fecal Endogenous Calcium and Phosphorus and True Digestibility. Endogenous fecal calcium and phosphorus was calculated by procedures described by Hansard *et al.* (1954b) using data from the concurrent chemical and radiochemical balance studies. These values (table 2) show that a significantly greater amount of fecal endogenous calcium was excreted by the control lambs ($P<.05$). Thus, daily fecal endogenous calcium excretion amount to 43.9 mg. per kilogram of body weight for the control lambs compared to 34.7 mg. for the lambs receiving stilbestrol. Fecal endogenous phosphorus excretion amounted to 61.9 mg. per kilogram and 57.7 mg. per kilogram for the control and stilbestrol-fed lambs, respectively, the difference not being significant.

Using these data for fecal endogenous loss, net absorption or true digestibility of the dietary minerals was calculated. True digestibility of calcium was not significantly different for the control lambs (45.1%) as compared to the stilbestrol-fed lambs (48.6%). For phosphorus, however, true digestibility was calculated to be 77.7% for the control lambs and 82.7% for the treated animals, a difference which approached significance at the .05 level.

The effects of stilbestrol upon calcium and phosphorus metabolism may be interpreted as growth stimulation. Dietary stilbestrol increased apparent digestibility of calcium without affecting true digestibility and reduced fecal endogenous calcium approximately 20%. Because urinary calcium excretion was negligible, the anabolic effects of dietary stilbestrol upon calcium metabolism may be attributed to a reduction in the net loss of the mineral from body stores.

Apparent digestibility of phosphorus, however, as well as its true digestibility was increased by stilbestrol feeding, and there was a slight reduction in fecal endogenous loss. Because urinary losses were unaffected by treatment, the increased retention resulting from stilbestrol apparently was the additive result of a moderate increase in absorption supplemented by a slight decrease in fecal endogenous phosphorus.

These data further indicate that the reduction in fecal endogenous calcium might be correlated with the increased phosphorus absorption. It may be postulated that calcium entered into combination with phosphorus in the plasma pool or at the site of bone mineralization, and thus aug-

mented the transport or the laydown of calcium in bone. However, the slight decrease in fecal endogenous phosphorus would indicate that the body itself was actively conserving minerals. Therefore, the role of stilbestrol might be considered as affecting calcium and phosphorus metabolism either by stimulating bone metabolism directly to induce mineral conservation, by permitting conditions for a more favorable calcium-phosphorus complex in the plasma pool or at the site of mineralization, or by a combination of these processes.

Summary

The response of lambs to stilbestrol feeding was measured using autoradiograph techniques as an indicator for bone growth. In addition concurrent chemical and radiochemical blood and balance studies were made with growing lambs to determine the effects of orally administered stilbestrol upon calcium and phosphorus metabolism. Results of these investigations indicated that in the ruminant animal the primary effect of stilbestrol administration was that of growth stimulation. This was manifested by a reduction in fecal endogenous calcium of approximately 20% with little apparent influence upon calcium absorption from the gastrointestinal tract. Phosphorus absorption was increased, but fecal endogenous phosphorus was only slightly decreased by the treatment.

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METHIONINE AND ANTIBIOTIC SUPPLEMENTATION FOR GROWING SWINE AT THREE PROTEIN LEVELS [1]

R. F. SEWELL AND B. C. KEEN, JR.
University of Georgia [2]

DATA are now available on the amino acid content of a wide variety of feed ingredients commonly used in practical swine rations (Lyman *et al.*, 1956; Williams *et al.*, 1955). Many investigations have also been conducted to determine the amino acid requirements of swine. Consequently, it is now possible to formulate swine rations giving due consideration to the assortment of amino acids supplied by the feedstuffs involved relative to the reported essential amino acid requirements for growing pigs.

It has also been shown that the protein allowances formerly prescribed for swine can be substantially reduced where the protein source is of high biological value and non-protein dietary factors are in adequate supply (Catron *et al.*, 1952; Burnside *et al.*, 1954).

Evidence has accumulated to indicate that essential amino acid requirement is related to level of protein in the diet. This general principle has been demonstrated in the case of the chick by Grau and Kamei (1950) for lysine and total sulfur amino acids and by Almquist (1949) for methionine. A similar relationship has been demonstrated with the pig in the case of lysine by Brinegar *et al.* (1950) and for isoleucine by Becker *et al.* (1957). This relationship suggests that the apportionment of amino acids in a feed mixture may warrant a more critical appraisal when protein level becomes the limiting condition in the diet.

The experiment reported here was conducted to investigate the effect of supplementing swine rations containing various levels of protein with methionine and an antibiotic.

Experimental Procedure

Sixty Duroc weanling pigs were stratified on the basis of weight and allotted to 12 groups of five pigs each. These groups were in turn allotted at random to a 2 x 2 x 3 factorial experiment in which the main treatment effects were antibiotic, methionine and protein level.

The rations were composed of ground yellow corn, solvent soybean oil meal, solvent peanut oil meal, limestone, defluorinated phosphate, salt, and

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vitamin supplements which contributed the following per lb. of ration: 4 mg. calcium pantothenate, 10 mg. choline chloride, 60 mcg. folic acid, 9 mg. niacin, 2 mg. riboflavin, 500 I.U. vitamin A and 100 I.U. vitamin D_2. A blend containing four parts soybean oil meal to one part peanut oil meal was used as the supplementary source of protein in all rations. Protein levels were calculated from chemical analysis of the ingredients used. The levels of protein used initially were approximately 20, 17, and 14%. These were adjusted downward to 17, 14 and 11% when the pigs averaged approximately 75 lb. and were decreased to 14, 11 and 8.4% when the pigs reached 125 lb. liveweight. The protein levels were obtained by adjusting the ratio of corn to the soybean-peanut oil meal blend in the ration.

The dl-methionine was added to those rations containing this supplement in amounts to adjust the total calculated methionine content to 2% of the dietary protein. Crystalline chlortetracycline was added at a level of 10 mg. per lb. of feed in those rations receiving this supplement.

All pigs were housed in a barn with concrete-floored pens and had free access to outside concrete runs. Feed and water were provided *ad libitum*. Individual weights and group feed consumption were recorded at 2-week intervals. Duration of the experiment was 79 days.

Results and Discussion

A summary of the average performance data are presented in table 1. The rate of gain data were analyzed by analysis of variance and a significant difference due to treatment was found. Comparison of the treatment means revealed a highly significant increase in rate of gain due to antibiotic supplementation. A significant difference due to protein level combination was also obtained. The 14–11–8.4% protein combination produced significantly slower gains than did the other protein combinations. No difference was found between the other two levels of protein. There was no significant response to methionine supplementation.

Statistical analysis revealed no significant interaction between either antibiotic and protein level or methionine and protein level. No significant interaction between antibiotic and methionine and protein level was found.

A study of the data presented in table 1 shows that the pigs receiving the 14–11–8.4% protein combination in the presence of chlortetracycline gained as rapidly as pigs receiving the higher protein combinations with or without chlortetracycline. This is in agreement with previous reports by Catron *et al.* (1952), Hoefer *et al.* (1952), and by Jensen *et al.* (1955). While there was no statistically significant interaction between protein level and antibiotic, it is interesting to note that there was a greater response to the antibiotic at the lower protein level than at the higher protein levels on those rations not containing methionine.

Grau and Kamei (1950) pointed out that increased amino acid requirement at higher protein levels does not necessarily mean that a definite percentage of the amino acid must be maintained in the dietary protein.

Becker *et al.* (1957) have presented data showing that the requirement for isoleucine by the pig is apparently not a fixed percentage of the dietary protein. In earlier work Becker *et al.* (1955) suggested that an indirect

TABLE 1. SUMMARY OF AVERAGE PERFORMANCE DATA OF PIGS FOR THE ENTIRE EXPERIMENT

Protein level	20	17	14	
	17	14	11	
Combination, %	14	11	8.4	Mean
Basal ration				
No. of pigs	5	5	5	15
Av. initial wt., lb.	46.8	45.0	45.0	45.6
Av. final wt., lb.	178.0	188.0	155.0	173.7
Av. daily gain, lb.	1.66	1.80	1.40	1.62
Feed per lb. gain, lb.	3.63	3.62	3.89	3.71
Chlortetracycline				
No. of pigs	5	5	5	15
Av. initial wt., lb.	46.8	46.4	45.6	46.3
Av. final wt., lb.	190.0	191.0	193.0	191.3
Av. daily gain, lb.	1.81	1.83	1.87	1.84
Feed per lb. gain, lb.	3.59	3.58	3.61	3.59
Methionine				
No. of pigs	5	5	5	15
Av. initial wt., lb.	46.8	45.8	45.0	45.9
Av. final wt., lb.	182.6	175.4	163.0	173.7
Av. daily gain, lb.	1.72	1.64	1.49	1.62
Feed per lb. gain, lb.	3.79	3.57	3.86	3.74
Chlortetracycline + methionine				
No. of pigs	5	4[a]	5	14
Av. initial wt., lb.	46.0	43.8	47.0	45.6
Av. final wt., lb.	197.0	195.0	179.0	190.3
Av. daily gain, lb.	1.91	1.91	1.67	1.82
Feed per lb. gain, lb.	3.68	3.72	3.62	3.67
Mean				
No. of pigs	20	19	20	
Av. initial wt., lb.	46.6	45.3	45.7	
Av. final wt., lb.	186.9	187.4	172.5	
Av. daily gain, lb.	1.77	1.80	1.61	
Feed per lb. gain, lb.	3.72	3.62	3.75	

[a] Pig died of undetermined cause. Value calculated by missing plot technique.
$s_{\bar{x}} = 0.109$.

relationship between the sulfur-bearing amino acids and protein level of the diet may exist. However, Sheffy (1955) reasoned that the requirements for the various amino acids at the different stages of growth may not vary markedly when expressed as a percentage of the dietary protein, considering the decreasing protein needs of the pig with increasing age and weight while there is apparently little difference in the tissue content of amino acids at the different stages of growth as shown by the studies of Williams *et al.* (1954).

Consequently, dl-methionine was added at levels of 0.08, 0.05 and 0.02% to the rations containing initial levels of 20, 17 and 14% protein, respectively. This adjusted the calculated methionine content of each ration to provide 2% of the total dietary protein. This plan of supplementation was used so as to relate the methionine content of each diet more directly to the protein content. Becker *et al.* (1955) found the methionine-cystine requirement of the pig to be 3.33% of the dietary protein of which 40% of the total requirement could apparently be satisfied by cystine. Accepting these values, the methionine requirement would be approximately 2% of the dietary protein as used in this experiment.

Calculation of the amino acid apportionment of the rations used indicates that methionine-cystine would have been the limiting amino acids at the 20 and 17% protein levels while lysine tended to become the limiting amino acid at the lower protein levels since a greater proportion of the protein of the ration was made up with corn protein. Methionine actually increased slightly when expressed as a percentage of the dietary protein at the lower protein levels. Failure to obtain a response to methionine supplementation on these rations which contained high-protein oil meals which are generally recognized to be limiting in methionine content indicates that there is little need for methionine supplementation of practical swine rations.

Summary

A factorial experiment was used to measure the effect of supplementing swine rations containing various levels of protein with an antibiotic and/or methionine.

In the absence of antibiotic supplementation, a 17–14–11% protein combination from weaning to terminal weights produced a higher rate of gain than did a 14–11–8.4% protein combination. No difference was obtained between protein levels when chlortetracycline was added to the rations.

There was a marked increase in rate of gain as a result of antibiotic supplementation of the rations employed. Methionine supplementation was without effect.

The data revealed no interaction either of antibiotic and protein level or of methionine and protein level.

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THE EFFECT OF VALERIC ACID AND A COMBINATION OF VALERIC AND ISO-VALERIC ACIDS ON THE PERFORMANCE OF MILKING COWS [1]

C. A. Lassiter, R. S. Emery and C. W. Duncan

Michigan State University, East Lansing

El-Shazly (1952) showed that amino acids in the presence of rumen microorganisms yield volatile fatty acids including valeric and iso-valeric acids. Bryant and Doetsch (1955) observed that the organism *Bacteroides succinogenes* required certain straight and branched chain fatty acids including valeric and iso-valeric acids for growth. Bentley *et al.*, (1955) found that valeric and iso-valeric acids, as well as other short chain fatty acids, stimulated cellulose digestion by centrifuged rumen microflora *in vitro*. Huhtanen and Elliott (1956) failed to observe a stimulation of cellulose digestion *in vitro* by the addition of valeric and iso-valeric acids when rumen fluid was present. MacLeod and Murray (1956) confirmed the observations of the Ohio workers (Bentley *et al.*, 1955) and also found that valine, leucine and isoleucine could largely replace the valeric and iso-valeric acids. Klosterman and Bentley (1957) reported that the supplementation of beef cattle fattening rations with a combination of valeric acid, biotin and para-amino-benzoic acid increased the growth rate of steers in three out of four trials when the rations fed were adequate in protein. In only two of the trials did there appear to be any improvement in feed efficiency. Hungate and Dyer (1956) observed that the weight gains of steers fed a high wheat straw ration were not affected by the addition of valeric and iso-valeric acids to the ration. The appetite of the animals appeared to be stimulated by the acids. Lassiter *et al.*, (1958) observed that a combination of valeric and iso-valeric acids improved the growth rate of dairy heifers fed a corn cob roughage ration from 1.26 to 1.47 lb. per day.

The purpose of the present study was to evaluate valeric acid and a combination of valeric and iso-valeric acids in the rations of milking cows fed high quality forages.

Experimental Procedure

Fifteen lactating dairy cows (six Holsteins, six Jerseys and three Guernseys) were used in five replicates of a 3 x 3 Latin square design to evaluate the use of valeric and iso-valeric acids in milking cow rations. Each period was 28 days in length with one week being used as a pre-

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liminary period. All cows within any group were comparable in respect to breed, stage of lactation, milk production, body weight, and general health and condition. Alfalfa hay and corn silage were used as roughages. The hay was a good quality first cutting alfalfa and was fed free choice. All Holstein and Guernsey cows were fed 30 lb. of corn silage and the Jersey cows were fed 20 lb. daily.

TABLE 1. COMPOSITION OF THE EXPERIMENTAL RATION

Ingredient	Grain mixture 1	Grain mixture 2	Grain mixture 3	Hay	Silage
	lb.	lb.	lb.		
Ground shelled corn	1260	1260	1260		
Ground oats	400	400	400		
Soybean oil meal	300	250	233		
Trace salt[a]	20	20	20		
Dicalcium phosphate	20	20	20		
Valeric acid premix[b]	....	....	73		
Valeric-iso-valeric acid premix[c]	....	60	....		
Total	2000	2010	2006		
	Chemical analysis (%)				
Dry matter	88.5	86.7	87.5	88.2	25.9
Crude protein	15.9	15.6	14.9	13.3	2.0
Crude fiber	4.4	4.5	4.2	27.5	5.8
Ether extract	3.1	2.9	3.1	1.6	0.8
Nitrogen-free extract	60.7	59.4	61.1	38.6	16.2
Ash	4.4	4.3	4.2	7.2	1.1

[a] Salt plus iodine and cobalt. (20 oz. of cobalt sulfate and 6 oz. of potassium iodide per ton of salt).
[b] 27.3 gm. of valeric acid per pound of soybean oil meal premix.
[c] 25.0 gm. of valeric acid, 8.33 of iso-valeric acid per pound of soybean oil meal premix.

The grain mixtures shown in table 1 were fed according to the U.S.D.A. (Woodward *et al.*, 1940) rules of feeding grain during the first period, after accounting for the grain furnished by the corn silage (Huffman and Duncan, 1954). The grain feeding rate was decreased according to the plan outlined by Lucas (1943) after the first period. Daily milk and feed weights were recorded. Milk samples were taken from four milkings each week for butterfat analysis. Body weights were recorded for three consecutive days at the beginning and at the end of each 28 day period. Grain Mixture 1 (table 1) was unsupplemented, Mixture 2 contained a combination of valeric and iso-valeric acids, and Mixture 3 contained only valeric acid.

Standard digestion and nitrogen balance trials were conducted on the experimental grain rations using four dry cows. All cows were fed 20.0 lb. of a first cutting alfalfa hay and 8.0 lb. of the experimental grain ration per day.

TABLE 2. EFFECT OF VALERIC ACID AND A COMBINATION OF VALERIC AND ISO-VALERIC ACID ON THE MILK PRODUCTION AND BODY WEIGHT CHANGES OF DAIRY COWS

Ration	Av. butterfat test	4% FCM[a]		Av. body weight change[a]
		Av. 7–28 days	Last 7 days	
	%	lb./day	lb./day	lb./cow/period
Control	5.0	35.5	34.8	−2.0
Valeric acid plus iso-valeric acid	4.8	35.5	34.9	9.3
Valeric acid	4.9	36.6	35.9	10.4

[a] No significant difference among rations.

Results and Discussion

The effect of the supplements on milk production, butterfat test and body weights are shown in table 2. Valeric acid alone and the combination of valeric and iso-valeric acids showed a slight tendency to depress the percentage of fat in the milk, but this difference was not statistically significant. Neither of the supplements had a significant effect on the production of 4% FCM even though the average production of the cows fed valeric acid alone was slightly higher than either the cows on the control ration or those on the ration containing the combination of acids. Cows fed the control ration lost in body weight slightly while cows fed both rations containing acids increased slightly in body weight. None of these body weight differences were statistically significant.

As shown in table 3, there was very little difference in the feed consumption of the various groups. Cows fed the ration containing the combination of valeric and iso-valeric acids as an average consumed slightly less hay than the other two groups. These differences in feed consumption were not statistically significant.

When comparing the results of this study with those reported recently (Lassiter *et al.*, 1958) with growing dairy heifers, several differences appear to exist. The growth stimulus in heifers observed from the feeding of a combination of valeric and iso-valeric acids may or may not express itself as a stimulus of milk production. The authors are not aware of any studies concerning the use of the so-called "rumen factors" in the rations of

TABLE 3. EFFECT OF THE VARIOUS EXPERIMENTAL RATIONS ON THE CONSUMPTION OF FEED (LB./COW/DAY)

Ration	Hay[a]	Corn silage	Grain[a]
Control	20.2	26.0	8.5
Valeric acid plus iso-valeric acid	18.8	26.0	8.7
Valeric acid	20.2	26.0	8.7

[a] No significant difference among rations.

lactating animals. Another difference could be in the type of roughage fed. In the trial with growing heifers, corn cobs were used as the sole source of roughage, whereas in the present study, a higher quality roughage was employed, alfalfa hay and corn silage. The daily intake of the acids was not the same in both studies. In the earlier trial, the heifers consumed 3.6 gm. of valeric acid and 1.0 gm. of iso-valeric acid per day. In terms of intake per 1,000 lb. of body weight, this was 4.56 gm. of valeric and 1.27 gm. of iso-valeric acids. In the present study, the average daily intake

TABLE 4. EFFECT OF THE VARIOUS EXPERIMENTAL RATIONS ON THE COEFFICIENTS OF APPARENT DIGESTIBILITY AND NITROGEN RETENTION OF DRY COWS

Comparisons	Ration 1	Ration 2	Ration 3	L.S.D.—5%
Coefficients of apparent digestibility (%) [a]				
Dry matter	68.3	68.9	71.6	1.1
Organic matter	69.7	69.7	72.8	1.2
Crude protein	76.4	74.2	76.1	n.s.
Ether extract	59.2	62.6	60.2	n.s.
Crude fiber	56.0	56.6	61.0	1.8
N-free extract	77.2	78.5	80.4	1.1
Nitrogen balance				
Daily intake (gm.)	313	311	307	n.s.
Daily output in feces (gm.)	73.8	80.0	73.5	n.s.
Daily output in urine (gm.) [b]	156.2	138.0	138.0	6.2
Daily retention (gm.)	83.0	93.0	95.5	n.s.
Percent retained of intake	26.5	29.9	31.1	1.9

[a] Coefficients for entire ration of 20.0 lb. of alfalfa hay and 8.0 lb. of grain mix.
[b] Missing plot values calculated for urine values for ration 3.

of valeric acid was 5.22 gm. and 2.18 gm. of iso-valeric acid when fed in combination. This represents an average daily intake per 1,000 lb. of body weight of 5.09 gm. of valeric and 2.13 gm. of iso-valeric acid. When valeric acid was fed alone, the average daily intake was 8.64 gm. and 8.43 gm. per 1,000 lb. of body weight. These calculations show that the daily intake of acids was higher in the present study. The optimum level of these acids in dairy cattle rations is not known.

The results of the digestion and nitrogen balance trials are shown in table 4. The grain ration containing the valeric acid alone significantly increased the digestibility of dry matter, organic matter, crude fiber and nitrogen-free extract. The combination of acids did not appear to influence greatly the digestibility of these nutrients. The grain ration containing valeric acid alone or in combination with iso-valeric acid significantly decreased the excretion of nitrogen in the urine and increased significantly the percent nitrogen retained of the intake and increased appreciably the total retention of nitrogen. These data show that these acids were affecting

nitrogen metabolism primarily by reducing the excretion of nitrogen in the urine.

Summary

Fifteen lactating dairy cows were used in a Latin square design to evaluate the effect of valeric and iso-valeric acids on the performance of milking cows. A good quality first cutting alfalfa hay and corn silage were used as sources of roughage.

The feeding of valeric acid alone or in combination with iso-valeric acid did not have any significant effect on milk production, body weight, butterfat percentage of milk, or the consumption of feed.

Digestion and nitrogen balance trials with dry cows showed that valeric acid alone increased the digestibility of dry matter, organic matter, crude fiber and nitrogen-free extract. Valeric acid alone or in combination with iso-valeric acid decreased the amount of nitrogen excreted in the urine and increased total retention of nitrogen as well as percent nitrogen retained of the intake.

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THE EFFECT OF BLEEDING VERSUS PREVIOUS INFECTION ON THE RESISTANCE OF LAMBS TO SUBSEQUENT INFECTIONS OF *HAEMONCHUS CONTORTUS* [1]

W. J. BEMRICK,[2] R. J. EMERICK,[3] R. F. SHUMARD,[4] A. L. POPE, AND P. H. PHILLIPS [5]

University of Wisconsin [6]

SEVERAL workers, including Stoll (1929), Ross (1932), Taylor (1934), Steward and Gordon (1953) and Steward (1955), have shown that sheep which have recovered from stomach worm infections are more resistant to reinfection by the same parasite than are previously uninfected animals. Fourie (1931), Andrews (1942) and Richard *et al.* (1954), noted that many of the changes in the various blood constituents due to stomach worm infection could also be produced by bleeding the sheep. This experiment was conducted to investigate the effects of bleeding on the ability of lambs to resist a heavy challenge infection of *Haemonchus contortus*.

Procedure

Eighteen lambs of predominantly Hampshire-Suffolk breeding were used on this experiment. The lambs were weaned at 2 months of age and divided into three lots of 6 lambs each. The twin lambs were assigned to different lots. All of the animals were full-fed a ration consisting of ground yellow corn and mixed grass-legume hay. Steamed bone meal was added as 1% of the grain ration and the animals were given free access to trace mineralized salt.

During the initial treatment period Lot I served as the control group, Lot II was infected with stomach worms by drenching each lamb orally with 20,000 infective *Haemonchus contortus* larvae and Lot III was periodically bled by jugular puncture, in an attempt to simulate the average hemoglobin level observed in the infected animals. The amount of blood removed was varied in the individual lambs in the group at each bleeding period in order that the hemoglobin values in the bled and infected lot might be maintained at a comparable level. When the hemoglobin values and fecal egg counts showed that the peak of infection had been

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[2] Present address: School of Veterinary Medicine, University of Minnesota, St. Paul 1, Minn.

[3] Present address: Station Biochemistry, South Dakota State College, Brookings, South Dakota.

[4] Present address: Eli Lilly and Co., Indianapolis, Ind.

[5] The authors wish to acknowledge the contribution in this study of Dr. C. A. Herrick, now deceased.

[6] Animal Husbandry, Biochemistry, Zoology and Veterinary Science.

reached, the amount of blood drawn was gradually reduced. This allowed the hemoglobin values of Lots II and III to remain comparable throughout the first phase of the experiment.

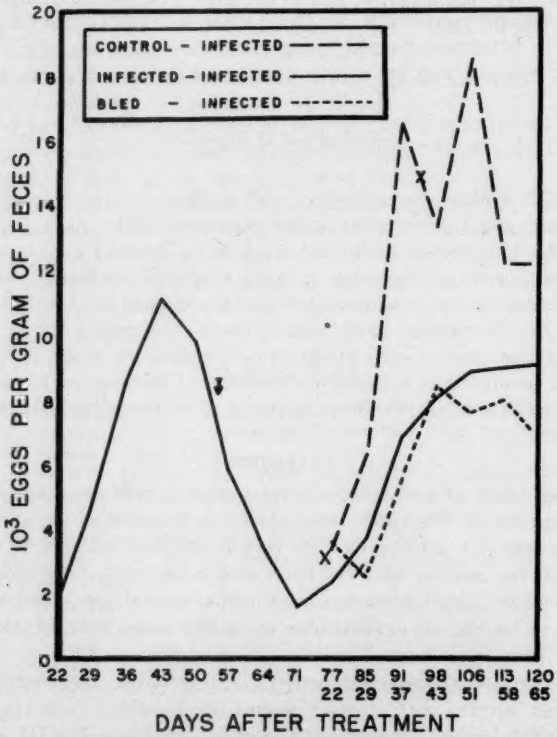

Figure 1. The effect of bleeding and stomach worm infection on the fecal egg counts of lambs during a subsequent parasite infection.
↓ Challenge infection administered.
× represents a death due to haemonchosis.

On the 55th day after the initial infection, when the lambs in Lots II and III appeared to be recovering, the second phase of treatment was started. All of the lambs in each lot were given a challenge infection consisting of 100,000 third stage *Haemonchus contortus* larvae. The response of the lambs to the challenge infection was followed by periodically determining fecal egg counts using the Stoll Technique (Stoll, 1930) and the hemoglobin values by using the oxyhemoglobin method (Hawk *et al.*, 1947). Weight gains, slaughter data and post-mortem worm counts were obtained.

Results and Discussion

A total of 3 deaths occurred as a result of infection. Two deaths in Lot I (control) and one death in Lot III (bled) occurred, respectively, 21, 41 and 28 days after the challenge infection.

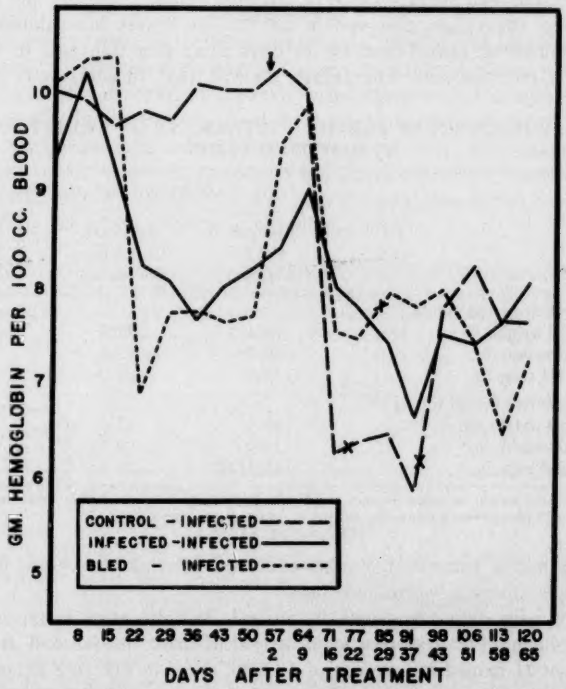

Figure 2. The effect of bleeding and stomach worm infection on hemoglobin values of lambs during a subsequent parasite infection.
↓ Challenge infection administered.
× represents a death due to haemonchosis.

The average number of parasite eggs found in the feces of the infected lambs are shown in figure 1. The average egg counts of the lambs in Lot I (control) reached a peak of 18,550 eggs per gram of feces. Lots II (infected) and III (bled) had consistently lower egg counts, reaching peaks of 9,300 and 8,440 eggs per gram, respectively. These data indicate that a previous stomach worm infection and bleeding by jugular puncture were similarly effective in reducing the fecal egg counts obtained during a subsequent stomach worm infection.

The hemoglobin data presented in figure 2 shows that the challenge infection resulted in the development of anemia in all of the lots. The anemia was initially more severe in the controls (Lot I) than in either the previously infected or the previously bled lambs.

During the period 16 to 37 days after the challenge infection, Lots II and III maintained average blood hemoglobin values 1 to 2 gm. per 100 c.c. higher than the values observed in Lot I. The lowest hemoglobin values were obtained in Lots I and II 37 days after infection and in Lot III 58 days after infection. The results showed that bleeding and previous

TABLE 1. THE EFFECT OF PREVIOUS TREATMENT ON WEIGHT GAINS OF PARASITIZED LAMBS

	Lot number and treatment		
	Control Lot I (6 lambs)	Infected Lot II (6 lambs)	Bled Lot III (6 lambs)
First treatment period (55 da.)			
Av. initial weight, lb.	35.1	38.0	40.3
Av. final weight, lb.	60.7	63.0	67.3
Av. weight gain, lb.	25.6	25.0	27.0
Second treatment period (68 da.)			
Av. initial weight, lb.	60.7	63.0	67.3
Av. final weight, lb.[a]	74.0	80.5	83.5
Av. weight gain, lb.	13.3(2)	17.5	16.2(1)

[a] Average final weight includes the last weight obtained for each animal before death or slaughter. Numbers in parentheses indicate the number of deaths due to haemonchosis.

infection had a somewhat similar effect on hemoglobin values during a subsequent stomach worm infection.

Weight gain data are shown in table 1. Weight gains were similar in all lots during the first treatment period. During the second treatment period Lot II gained an average of 17.5 lb. and Lot III 16.2 lb. per lamb. These figures represent average increases of 4.2 and 2.9 lb., respectively, over the 13.3 lb. gained by Lot I during the same period. These weight gains were relatively low for all lots and the differences were not statistically significant.

All of the surviving animals were sacrificed and carcass grades and stomach worm counts were obtained at the termination of the experiment. The carcass grades are presented in table 2. An average carcass grade of low choice was obtained in the bled and the previously infected lots. This was one grade higher than the average grade of low good obtained in the control lot. It is of further interest that the only two lambs that graded prime were found in Lot III (bled).

The abomasum of the lambs were examined to obtain total worm counts. The average worm count of Lots I, II, and III were 2,930, 3,025 and 3,305,

respectively. The results indicate that a previous infection or bleeding had no significant effect on the number of worms comprising a subsequent infection. These counts were obtained at the termination of the experiment, however, and are not indicative of the number of worms present at the peak of infection.

Summary

An experiment was conducted to investigate the effect of bleeding on the development of resistance to stomach worm infection in sheep. Under the conditions of this experiment a previous infection with *Haemonchus contortus* and bleeding permitted the development of similar degrees of resistance to a subsequent infection with the same parasite. This suggests that one of the most important factors in the development of a resistance in lambs to challenge infections with *Haemonchus contortus* is the hemorrhage produced by the blood sucking habits of the worms.

TABLE 2. CARCASS GRADE AND DEATH DISTRIBUTION OF PARASITIZED SHEEP SUBJECTED TO DIFFERENT TREATMENTS PRIOR TO INFECTION

	Prime			Choice			Good			Utility			
Treatment	+	Av.	−	+	Av.	−	+	Av.	−	+	Av.	−	Deaths[a]
Control/infected				1		1	2						2
Infected/infected				3	1				1	1			
Bled/infected		1	1		1	1	1						1

[a] Deaths were due to haemonchosis.

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THE COMPARATIVE FEEDING VALUE OF HIGH MOISTURE CORN AND LOW MOISTURE CORN WITH DIFFERENT FEED ADDITIVES FOR FATTENING BEEF CATTLE [1]

W. M. Beeson and T. W. Perry

Purdue University Agricultural Experiment Station [2]

PRESERVING high-moisture ground ear corn (32% water) by storing it in an air-tight glass-lined silo has introduced a new method of processing corn for cattle feeding. Many times corn is too high in moisture content to be stored safely in a crib without artificial drying. The ensiling of such corn offers a process for preserving the corn without deterioration or appreciable loss of feeding value.

The objectives of the research reported in this paper were:

(1) To compare the relative feeding value of ensiled ground ear corn (high-moisture) with regular ground ear corn produced from the same field, and

(2) To study the effect of the addition of oxytetracycline (Terramycin), a primary grown yeast processed from wood sugars (Torula Yeast), and tetra-alkylammonium stearate or TAAS (Dynafac).

Materials and Methods

Trial I. Two-year old steers which had been wintered previously on ground corn cobs and Purdue Supplement A and then either fed green chopped forage or pastured during the summer were used for this study. Forty head of steers were divided into 4 lots of 10 steers each on the basis of liveweight and apparent thrift. A control ration of a full feed of ground ear corn, 3.7 lb. Purdue Supplement A and free choice minerals was fed. The treatments were as follows: Lot I, regular ground ear corn; Lot II, high-moisture ground ear corn; Lot III, regular ground ear corn plus 100 mg. oxytetracycline per steer daily; Lot IV, high-moisture ground ear corn plus 100 mg. oxytetracycline per steer daily.

Both types of ground ear corn were fed twice daily (morning and evening) according to the appetite of the steers. No hay or other roughage was fed during the experiment, excepting the roughage that was provided in the form of ground cobs in the ground ear corn. The mineral mixture was composed of two parts of bonemeal and one of salt, which was fortified with one ounce of cobalt sulfate per 100 pounds of salt. Also, in another box, the steers had free access to plain salt.

[1] The authors express sincere appreciation to the A. O. Smith Corporation, Milwaukee, Wisconsin for furnishing the glass-lined silo for this research.

[2] Contribution from the Department of Animal Husbandry, Journal Paper No. 1173, Purdue University, Lafayette, Indiana.

All of the corn was harvested from the same field so that the two types of corn would have essentially the same origin. The high-moisture corn was picked with a mechanical corn picker on September 20 and 21, 1955, and immediately ground through a hammer mill and blown into an airtight silo. At the time of harvest the corn was mature, fully dented and contained 32.2% moisture. The glass-lined silo was sealed and not opened until November 2. For each feeding the ground ear corn was unloaded from the bottom of the silo with a mechanical unloader. There was no spoilage in the corn, but it had gone through the typical fermentation process and had an excellent aroma.

The regular ear corn was picked on October 17 and 18, 1955, and stored in a crib with a moisture content of 17.7%. There was no spoilage. Ear corn was ground through a hammer mill using a ½-inch screen and essentially the same "grind" as used for high-moisture corn.

Trial II. Six lots of 12 heifer calves each were fed a control ration of a full feed of ground ear corn, 3.0 lb. Purdue Supplement A, free choice mixed hay, and free choice minerals. Lots I, II, and III were fed high moisture ground ear corn and Lots IV, V, and VI were fed regular ground ear corn. Lots I and IV served as controls; Lots II and VI were fed 0.10 lb. of wood yeast per heifer daily, and Lots III and VI were fed 200 mg. TAAS (diluted on 800 mg. bone meal) per heifer daily.

Both types of ground ear corn were fed twice daily (morning and evening) according to the appetites of the heifers in each lot. Hay allowance was gradually reduced as the feeding period progressed from 5 lb. per head daily at the beginning to 2 lb. for the last 6 weeks. The same type of mineral feeding referred to in Trial I was followed.

The high-moisture corn was picked with a mechanical picker on September 26 to 28, 1956, and processed as described in Trial I. The high-moisture ground ear corn contained 32.5% moisture. The regular ear corn was picked from October 25 to November 1, and contained 15.5% moisture.

Results

Trial I. When high-moisture corn (32.2%) was converted to the same moisture basis as the regular ground ear corn (17.7%) there was from 12 to 15% saving in feed. Both types of corn were palatable and the dry matter intake of the two types of corn was very close. No difficulty was encountered in keeping the cattle on feed.

Feeding 100 mg. oxytetracycline per steer daily, either with regular or high-moisture corn, did not improve daily gain or feed efficiency.

Although the steers fed the high-moisture corn gained slightly faster (0.13 lb. to 0.23 lb. more per day) these differences were not statistically significant.

Trial II. On the same moisture basis (15.5%) heifers fed on the high-moisture ground ear corn required 10% less corn to produce a pound of gain than heifers fed on regular ground ear corn.

The feeding of either wood yeast or tetra-alkylammonium stearate had no consistent effect on growth rate or feed efficiency.

Discussion

The data from the two trials reported in this paper indicate fattening beef cattle (steers, Trial I; heifers, Trial II) make more efficient utiliza-

TABLE 1. A COMPARISON OF HIGH-MOISTURE GROUND EAR CORN VS. REGULAR GROUND EAR CORN, WITH AND WITHOUT ANTIBIOTICS FOR FATTENING STEERS (NOVEMBER 2, 1955–FEBRUARY 27, 1956, 117 DAYS) TRIAL I

	No antibiotic		100 Mg. oxytetracycline per steer daily	
	Regular corn	High-moisture corn	Regular corn	High-moisture corn
Moisture content of corn, %	17.7	32.2	17.7	32.2
Number of animals	10	10	10	10
	Growth data			
Initial wt., lb.	960	958	960	930
Final wt., lb.	1232	1257	1234	1218
Total gain, lb.	272	299	274	288
Daily gain, lb.	2.33	2.56	2.34	2.47
	Daily feed			
Ground ear corn, lb.	22.1	25.0(20.6)[a]	23.1	26.0(21.4)[a]
Supplement A, lb.[b]	3.7	3.7	3.7	3.7
	Feed per 100 lb. gain			
Ground ear corn, lb.	951	979(807)[a]	988	1052(866)[a]
Supplement A, lb.	159	145	159	151
Corn saved, %	...	15	...	12

[a] These figures represent the amount of ground ear corn consumed and required per 100 lb. gain when converted to the same moisture basis (17.7%) as the regular ground ear corn.

[b] Formula for Purdue Supplement A (1,000 lb. mix): soybean oil meal, 650 lb.; dehydrated alfalfa meal, 140 lb.; molasses, 140 lb.; bone meal, 52 lb.; salt with cobalt, 17 lb.; vitamin A & D concentrate, 0.5 lb. (4,540,000 U.S.P. units Vitamin A and 567,500 U.S.P. units Vitamin D per lb.)

tion of corn which is harvested when it contains from 32 to 33% of moisture and which is ensiled in airtight storage until fed. The increased efficiency of utilization ranged from 10 to 15%. Subsequent research data reported by Culbertson *et al.* (1957) confirm the results reported in this paper in which a feed saving of 10% was obtained by feeding high-moisture ground ear corn (31% moisture) over feeding regular ground ear corn (14% moisture) to fattening beef cattle. The results of one trial with swine (Conrad and Beeson, 1956) indicated swine utilize high-moisture shelled corn approximately 8% less efficiently than regular shelled corn when the utilization of the high-moisture shelled corn (31.8% moisture) was converted to the same moisture content of the regular shelled corn

TABLE 2. WOOD YEAST AND TAAS AS ADDITIVE TO HIGH AND LOW MOISTURE GROUND EAR CORN FOR FATTENING HEIFER CALVES DECEMBER 6, 1956 TO APRIL 11, 1957. 126 DAYS) TRIAL II

	High-moisture gr. ear corn 32.5% moisture				Regular gr. ear corn 15.5% moisture			
	Lot 1 Control	Lot 2 Wood yeast	Lot 3 TAAS	Av.	Lot 4 Control	Lot 5 Wood yeast	Lot 6 TAAS	Av.
No. of animals	12	12	12		12	11	12	
			Growth data					
Initial, wt., lb.	466	469	465	467	466	465	467	466
Final, wt., lb.	720	744	745	736	749	743	730	741
Total gain, lb.	254	275	280	269	283	278	263	275
Daily gain, lb.	2.02	2.18	2.22	2.14	2.24	2.20	2.09	2.18
			Daily feed					
Gr. ear corn, lb.	14.61(11.78)[a]	14.88(11.89)[a]	15.00(11.98)[a]	14.87(11.88)[a]	13.52	13.29	13.54	13.45
Supplement A, lb.	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Hay, lb.	3.49	3.59	3.50	3.53	3.69	3.70	3.85	3.75
Wood yeast, lb.	.....	.10	.....	.....	.....	.10	.....	.....
TAAS, grams	.....	.....	1.0	.....	.....	.....	1.0	.....
			Feed per 100 lb. gain					
Gr. ear corn, lb.	730(583)[a]	682(545)[a]	675(539)[a]	695(555)[a]	602	603	648	617
Supplement A, lb.	148	137	135	140	134	136	144	138
Hay, lb.	173	164	157	165	164	158	184	172
Corn saved, %	...	...	...	10	...	...	...	...

[a] These figures represent the amount of ground ear corn consumed and required per 100 lb. gain when converted to the same moisture (15.5%) basis as regular ground ear corn.

(18.5% moisture). Although it is difficult to compare the results obtained from feeding ground ear corn to cattle with those from feeding shelled corn to swine, it is reasonable to suggest beef cattle make better utilization of the cob portion of the high-moisture ground ear corn than of regular ground ear corn.

No growth stimulation was obtained from feeding an antibiotic, a wood yeast, or a tetra-alkylammonium stearate (TAAS) compound. The effect of feeding antibiotics on high corn diets has not been definitely established. Under certain feeding conditions a growth stimulation has been obtained even on high energy diets, and this stimulation was over that obtained through the use of either orally administered or subcutaneously implanted diethylstilbestrol (Perry *et al.*, 1957). However, on limited grain intake or high roughage diets, the feeding of antibiotics consistently increases gain and improves the efficiency of feed utilization of beef cattle (Perry *et al.*, 1954).

Culbertson *et al.* (1957) obtained no increase in rate of gain by feeding two grams of a 20% TAAS compound with either high moisture or regular ground ear corn with and without 10 mg. of diethylstilbestrol per day. Kolari *et al.* (1957) likewise obtained no effect from the feeding of 2 gm. of the 20% TAAS compound, per head daily, in a fattening ration to steers weighing 800 pounds at the start of a 112 day fattening trial. Beeson *et al.* (1953) obtained a statistically significant increase in the rate of gain of swine by including 0.20% of TAAS.

In contrast to no growth stimulation obtained by feeding wood yeast, under the conditions of this trial, Burroughs and Culbertson (1957) reported a slight increase in gain (2.51 lb. vs. 2.43 lb. per day) by the feeding of a small amount of wood yeast.

Summary

The results of two trials indicate fattening beef cattle (two year old steers and heifer calves) utilize high-moisture ground ear corn (32% moisture) from 10 to 15% more efficiently than regular ground ear corn, when the high-moisture corn is converted back to the same moisture content as the regular ground ear corn.

No consistent growth stimulation was obtained by the addition of oxytetracycline, a wood yeast or tetra-alkylammonium stearate, to either the high-moisture corn or the regular ground ear corn rations.

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Beeson, W. M., T. W. Perry and P. J. Reynolds. 1953. The effect of surfactants on the growth rate of swine. J. Animal Sci. 12:619.

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Initial, wt., lb.	466	469	465	467	466	465	467	466
Final, wt., lb.	720	744	745	736	749	743	730	741
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Daily gain, lb.	2.02	2.18	2.22	2.14	2.24	2.20	2.09	2.18
			Daily feed					
Gr. ear corn, lb.	14.61(11.78)[a]	14.88(11.89)[a]	15.00(11.98)[a]	14.87(11.88)[a]	13.52	13.29	13.54	13.45
Supplement A, lb.	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Hay, lb.	3.49	3.59	3.50	3.53	3.69	3.70	3.85	3.75
Wood yeast, lb.	.....	.10	.....	.....	.....	.10	.....	.....
TAAS, grams	.....	.....	1.0	.....	.....	.....	1.0	.....
			Feed per 100 lb. gain					
Gr. ear corn, lb.	730(583)[a]	682(545)[a]	675(539)[a]	695(555)[a]	602	603	648	617
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[a] These figures represent the amount of ground ear corn consumed and required per 100 lb. gain when converted to the same moisture (15.5%) basis as regular ground ear corn.

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EFFECTS OF ADMINISTRATION OF OILS AND OF PENICILLIN ON INCIDENCE AND SEVERITY OF BLOAT AND CERTAIN OTHER RESPONSES OF CATTLE[1]

L. R. Brown, R. H. Johnson, N. L. Jacobson and P. G. Homeyer

Iowa State College, Ames

IT is difficult to estimate the extent of direct and indirect losses due to bloat, but there is no doubt that these losses are great and on occasion may be disastrous. In spite of exhaustive research, however, no completely successful preventive or therapeutic measure for bloat is presently available. Barrentine *et al.* (1956) reported that both procaine and potassium penicillin (50 mg. per animal) administered either by capsule or in an aqueous suspension as a drench to yearling steers grazing ladino clover gave protection for periods of 1.5 to 3 days. Thomas (1956) observed no severe bloating in cattle during the period from 12 to 96 hours after each animal had been given 100 mg. of procaine penicillin. Untreated animals exhibited extensive bloat. During these studies no adverse effects of penicillin upon appetite were observed.

New Zealand workers (Johns, 1954; Reid, 1955, 1956; and Sears and Reid, 1955) prevented bloat by spraying pastures with an emulsified oil (usually peanut oil, although cream, tallow, and other vegetable oils were used) and limiting grazing to sprayed forage. Sears and Reid (1955) reported some success from adding unemulsified peanut oil to the drinking water, a procedure which is less laborious. However, the spraying method was believed to be more effective. These authors postulated that the preventive effect of the oils was due to the surface activity of oil in breaking foam. Johnson *et al.* (1956) reported less bloat in calves fed a pelleted starter containing 10% tallow. It was suggested that the high level of fat may have lowered surface tension and reduced foaming in the rumen. It is interesting to note that more than 2 decades ago Berkman and Egloff (1934) recommended the use of certain oils as foam preventives in various industrial processes.

The possibility of administering an oil in the drinking water appears promising, provided the oil is rendered water dispersible. Such a product was given major attention in the investigation reported herein. Also, other methods of administering oil and the use of penicillin seemed to warrant further attention. The present study was designed to examine the effects of various oils and of penicillin upon the incidence and severity of bloat, feed consumption, and weight gains. A preliminary report has been presented (Johnson *et al.*, 1956).

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Experimental

Experiment 1. Water Dispersible Oil in the Drinking Water of Cattle on Alfalfa Pasture and in Feed Lot

In a preliminary study with 12 young dairy heifers receiving alfalfa hay *ad libitum* and 4 lb. per day of a corn-oats-soybean oil meal mixture, a water-dispersible oil [2] was added to the drinking water (0.01% to 2.0% of the total liquid) for a period of 6 weeks. No apparent adverse effects upon feed and water consumption or upon weight gains could be attributed to addition of the oil.

Phase 1. Twenty-five Holstein steers (average initial weight 620 lb.) on alfalfa pasture were divided randomly into two groups. During a series of

TABLE 1. DESIGN OF EXPERIMENT I, PHASE 1

	Period							
	1		2		3		4	
	May 15 to May 28	May 29 to June 10	June 11 to June 24	June 25 to July 8	July 9 to July 22[a]	Aug. 9[a] to Aug. 22	Aug. 23 to Sept. 6	Sept. 7 to Sept. 15
Group 1	1% oil	Control	1% oil	Control	1% oil	Control	2% oil	Control
Group 2	Control	1% oil	Control	1% oil	Control	1% oil	Control	2% oil

[a] Pasture was exhausted due to drought. Animals were not on pasture from July 23 to Aug. 8.

9- to 15-day sub-periods in the spring and summer of 1956 each group alternately received water-dispersible oil in the drinking water or served as controls. Table 1 shows the experimental design. Initially the oil was fed at a level of 1% but it was increased to 2% as water consumption decreased with the advent of cooler weather and considerable rain in period 4. Throughout the daylight hours observations of bloating incidence and severity, grazing intensity, and water consumption were taken hourly. Forage quality and meteorological conditions were recorded daily. The animals were weighed at the beginning of each sub-period.

Results of this study are summarized in table 2. The mean maximum daily bloat was computed for each animal within each of the sub-periods and an analysis of variance (Snedecor, 1956) of these means was computed for each of the periods. The cross-over type of design used assumes negligible interactions among groups, treatments and sub-periods. The interaction of treatments with animals within groups was used as the estimate of error for treatments. The mean bloat for the animals on the oil treatment was significantly less than that for the control animals at $P=.01$ in periods 2 and 4 and at $P=.05$ in period 1. Although the mean difference in bloat favored the oil treatment in period 3, it was not significant at $P=.05$. Differences in mean bloat between the two groups of animals for the

[2] A lard derivative supplied by Midwest Dried Milk Co., Dundee, Illinois through the courtesy of R. F. Van Poucke.

four periods were extremely small, indicating no important real intergroup differences in bloating susceptibility.

Grazing intensity was estimated hourly by assigning to each animal a figure ranging from 0 (no grazing) to 2 (full grazing). Differences computed for the first two periods were very small and appeared to be of little significance, especially in view of the subjectivity of the method of estimation. Water consumption was consistently higher for the treated group. Estimated average daily oil intake per treated animal was 0.95, 1.56, 1.15, and 1.50 lb. during periods 1, 2, 3, and 4, respectively.

TABLE 2. SUMMARY OF EFFECTS OF WATER-DISPERSIBLE OIL ON BLOAT SEVERITY, FEED AND WATER CONSUMPTION, AND WEIGHT GAINS, EXPERIMENT I, PHASE 1

	Period							
	1		2		3		4	
Measurement	Oil	Control	Oil	Control	Oil	Control	Oil	Control
Bloat severity:								
Av./animal/day[a]	0.04	0.09	0.09	0.20	0.16	0.22	0.23	0.57
Av. max./animal/day[b]	0.35*	0.59	0.58**	0.87	0.78	0.91	0.99**	1.74
Grazing intensity[c]	0.53	0.58	0.76	0.73	....	....	....	....
Water consumption /animal/day, lb.	95	82	156	130	115	102	75	59
Av. daily wt. gains, lb.	3.73	3.47	1.55	0.68	0.51	—0.04	1.44	2.20

[a] Based on rating from 0 (no bloat) to 5 (severe bloat); average of all hourly observations per day.
[b] Average of maximum values for each animal.
[c] Estimate of grazing intensity (during first 8 weeks).
* Significant at P 0.05.
** Significant at P 0.01.

Average daily weight gains varied considerably from period to period and were greater for the treated groups in the first three periods, but not in the fourth. Average daily gains of the 14 animals for which weights were taken during all four periods were 1.76 and 1.52 lb. for the treated and non-treated animals, respectively.

Several rather heavy rains in late August and early September followed a period of drought. Subsequent to the rains the rapidly growing forage produced severe bloat. Oral administration of 1 lb. of oil to each of 10 animals in the late afternoon of Sept. 5 completely prevented bloat in the treated group (not fed oil in the water on this day) during evening grazing whereas seven of the 10 untreated animals bloated; e.g., two died from acute bloat and five others required treatment. It was necessary to remove the untreated animals from pasture during the night; treated animals were allowed to graze and no appreciable bloating was observed. At this point, treatment groups were switched and on subsequent days 2% oil in the drinking water prevented serious bloating. During the same period the untreated animals continued to bloat and were removed from pasture at night to prevent losses.

Both in this phase of experiment I and in phase 3 of experiment II some

very severe cases of bloat resulted. When these were observed, an attempt was made to relieve the animal by passing a stomach tube into the rumen to remove the gas. In most instances this procedure failed to bring adequate relief. When an animal failed to respond to the stomach-tube treatment, 100 ml. of the lard oil was injected intraruminally by means of a large syringe and needle. In 12 of 13 cases where the bloat was rated as 4

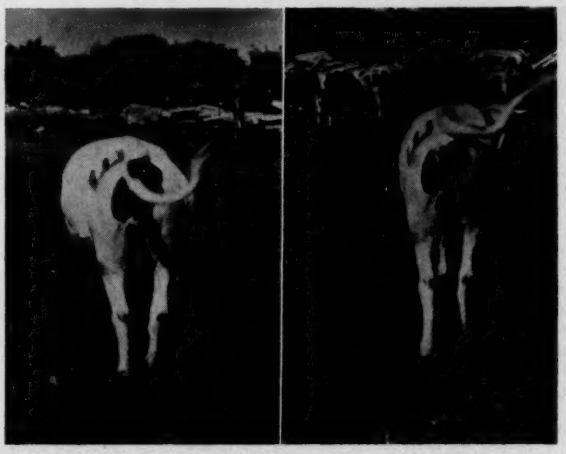

Figure 1. A typical case of alfalfa pasture bloat (left) and the same animal 15 minutes after intraruminal injection of 100 ml. of the water-dispersible oil (right).

or higher, an injection of oil apparently brought about a release of gas from the ingesta to the free gas space above it; the animals either began to eructate gas voluntarily in 15 to 40 minutes, or passage of a stomach tube brought release of large amounts of free gas, and the animals recovered. However, the possibility of spontaneous recovery cannot be ignored. One of the injected animals died, perhaps before the oil could take effect since the oil was injected when the animal appeared moribund. Figure 1 illustrates a typical case before and 15 minutes after treatment. The bloat in this case was typical of that rated as No. 4.

Phase 2. At the close of the 1956 pasture season the 18 steers remaining from the original 25 (four died and three were removed for other reasons) were confined to a feed lot and were fed medium quality alfalfa hay and a concentrate mixture consisting of 70% corn, 27% soybean oil meal, 1% calcium carbonate, 1% dicalcium phosphate, and 1% salt. When on full feed each animal consumed approximately 17 lb. per day of the concentrate plus 6.5 lb. of hay. The animals then were divided into two groups with an attempt being made to divide the most susceptible bloaters

evenly between the groups. Each group either received 1% oil in the drinking water or alternately served as control during three 10-day periods. Animals were fed at 6 a.m. and 4 p.m. Following the morning feeding, observations of bloating severity were taken hourly through 10 a.m. and in the afternoon at 4 and at 5 p.m. The animals were weighed at the beginning of each period. Three times during each period samples of rumen fluid were collected by stomach tube from an equal number of animals in each group.

Results of this phase are summarized in table 3. The incidence of feed-lot

TABLE 3. SUMMARY OF EFFECTS OF WATER-DISPERSIBLE OIL ON BLOAT SEVERITY, WATER CONSUMPTION, AND WEIGHT GAINS, EXPERIMENT I, PHASE 2

	Period					
	1		2		3	
Measurement	Oil	Control	Oil	Control	Oil	Control
Bloat severity:						
Av./animal/day[a]	0.08	0.25	0.17	0.12	0.15	0.17
Av. max./animal/day[a, b]	0.24	0.49	0.38	0.32	0.33	0.42
Water consumption /animal/day, lb.	82	79	89	86	76	72
Av. daily wt. gains, lb.	2.10	1.04	2.56	1.33	1.39	—0.97

[a] See footnotes in table 2.

[b] Analysis of variance was made over all periods. F was non-significant (at P .05) for treatments vs. animals within groups x treatments or for groups vs. animals within groups.

bloat was low and although the oil appeared to have a small beneficial effect this effect was not statistically significant at P=.05.

As in the pasture study water consumption was consistently higher for the treated group although the differences were smaller. On the basis of water consumption by the treated group and the oil level employed, the average daily oil intake of each treated animal during periods 1, 2, and 3 was 0.82, 0.89 and 0.76 lb., respectively. Average daily weight gains were consistently in favor of the treated group (2.01 lb. per day for treated animals versus 0.47 lb. for the controls).

Experiment II. Oral Administration of Procaine Penicillin to Cattle Fed Alfalfa Soilage and High-Concentrate Rations

Phase 1. Fifteen dairy animals (average initial weight 520 lb.) were employed to study the effects of oral administration of a penicillin feed supplement [3] (mixture of 50% procaine penicillin and 50% ground oyster shell meal). The animals were divided into two groups (based upon severity of bloat during a 3-day preliminary period) which appeared to be approxi-

[3] Supplied by Merck and Co., Rahway, N. J., through the courtesy of L. Michand.

mately equal in bloating potential. Prior to feeding, approximately 75 mg. of procaine penicillin were administered daily via gelatin capsule to each animal in the treated group. Groups alternately received penicillin and served as controls for three periods of 11, 10 and 11 days, respectively. An adjustment period of at least 7 days was allowed between the time a group received penicillin and the beginning of the control period.

TABLE 4. SUMMARY OF EFFECTS OF PENICILLIN ADMINISTRATION ON INCIDENCE AND SEVERITY OF BLOAT AND ON WEIGHT GAINS, EXPERIMENT II

Measurement	Phase 1					
	Period					
	1		2		3	
	Pen. (8)[c]	Control (7)	Pen. (7)	Control (8)	Pen. (8)	Control (7)
Bloat severity[a]	0.24	0.50	0.17	0.20	0.04	0.14
Bloat incidence[b]	19	36	13	11	3	11
Av. daily wt. gains, lb.	5.17	3.87	−0.92	0.42	2.38	2.66

	Phase 2		Phase 3	
	Pen. (7)	Control (8)	Pen. (7)	Control (8)
Bloat severity[a]	0.48	0.10	0.50	0.26
Bloat incidence[b]	30	4	34	20
Av. daily wt. gains, lb.	3.04	3.24	1.61	1.14

[a] Based on rating from 0 (no bloat) to 5 (severe bloat); average of all hourly observations per day.
[b] In percent.
[c] Number in parenthesis indicates no. of animals per group.

The capsules were prepared in lots of 100. To facilitate handling, one part of the penicillin supplement was mixed with 20 parts of glucose. A procaine penicillin assay (U. S. Department of Health, Education and Welfare, 1955 and Grove and Randall, 1955) indicated the presence of an average of 77±3 mg. of procaine penicillin per capsule.

The animals were confined in a single dry-lot and were fed fresh alfalfa soilage *ad libitum*. Any excess feed from the previous day was removed from the feed bunks before the next daily feeding at approximately 8 a.m. After feeding, hourly observations of bloat incidence and severity were taken. Animals were weighed at the beginning and end of each period; feed consumption and forage quality were recorded daily. Results are summarized in table 4.

The average bloat severity was less for animals administered penicillin than for the controls in all three periods, but only in period 1 was the difference statistically significant at $P=.05$. No appreciable effect upon weight gains was noted.

Phase 2. When the drought of mid-summer severely limited the supply of fresh alfalfa, the animals were fed *ad libitum* a high concentrate diet (the concentrate mixture described in phase 2 of experiment I) with a limited amount of alfalfa soilage (20 lb. per animal per day). The concentrate was fed twice daily, at 8 a.m. and 4 p.m. Penicillin was administered continuously to one group of animals by the method described in phase 1.

The larger mean bloat incidence for the penicillin-fed group was due mainly to frequent bloating of two animals in this group. Consequently

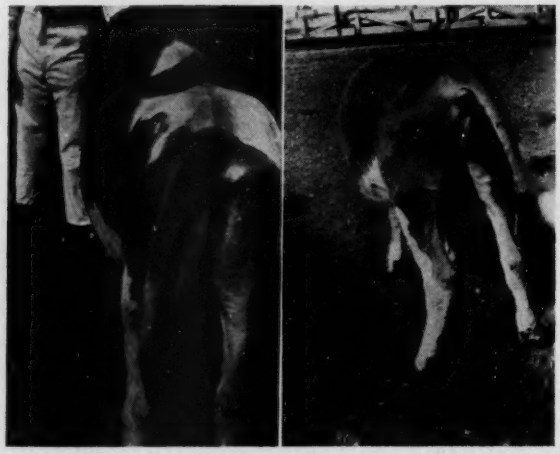

Figure 2. Animals bloated on alfalfa soilage; control (left), penicillin (right).

the experimental error was extremly large and the mean difference in bloat severity between the two groups was not statistically significant at P=.05.

Phase 3. After late summer rains produced an abundance of lush alfalfa the animals again were changed to *ad libitum* feeding of alfalfa soilage. The penicillin group from phase 2 continued to receive penicillin throughout this trial. More severe bloat occurred during this phase than was experienced in the earlier two phases. This increase in bloat was concurrent with the increase in bloat among animals in the pasture plots (experiment I, phase 1). During this 21-day period with the 15 animals there were 4 days when all animals, 2 days when 14 animals, 4 days when 13 animals, and 6 days when 12 animals bloated to some degree. The animals required constant observation for a considerable period of time after feeding; several were relieved via stomach tube and in three cases other treatment appeared necessary to prevent death. The results are summarized in table 4. Again the greater bloat severity for the penicillin-fed animals

was not statistically significant at P=.05. Typical bloat during this phase of experiment II is shown in figure 2.

Experiment III. Various Oils Mixed with Alfalfa Soilage Prior to Feeding

After the peak bloat severity in phase 3 of experiment II, three oils were applied, either by sprayer or sprinkling can, to the alfalfa prior to feeding.

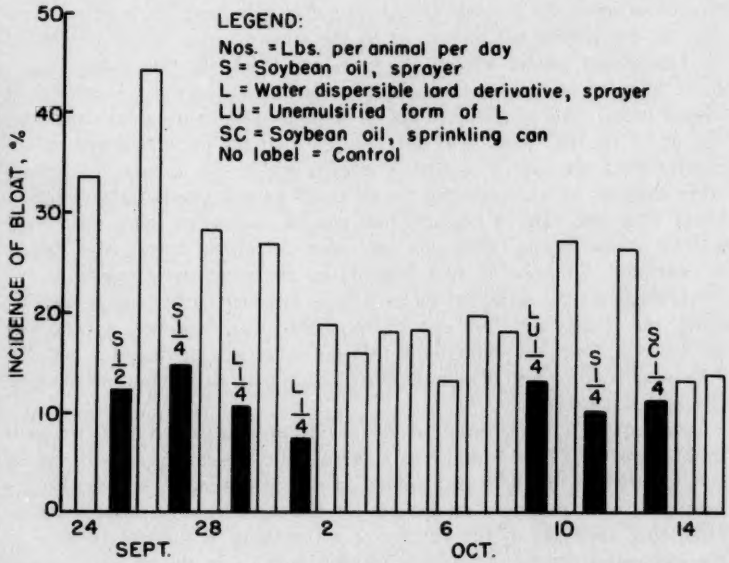

Figure 3. Effects of various oils (sprayed or sprinkled on alfalfa soilage) on incidence of bloat.

Soybean oil was applied first at the rate of 0.5 lb. per animal per day and appeared to reduce bloat markedly. It also seemed to be effective at the rate of 0.25 lb. per animal per day; therefore, in subsequent trials all oils were used at this level. The water dispersible lard oil used in experiment I and an unemulsified form of the same product also appeared to be effective at the 0.25 lb. level. In this study all animals received the oil on the days of treatment and penicillin administration was continued to the same group as in phase 3 of experiment II. The data are summarized in figure 3. In all cases when the forage was treated with oil a considerable reduction in incidence of bloat was noted. However, because of the limited nature of these observations, conclusions should be tentative only.

At frequent intervals throughout these studies samples of rumen fluid

were collected by stomach tube from equal numbers of animals in the treated and untreated groups. Results of analyses of these samples will be presented in a subsequent report.

Discussion

Results of this study show that addition of a water-dispersible oil to the drinking water greatly reduced the incidence and severity of pasture-type bloat when the animals consumed sufficiently high levels of oil. The data do not permit speculation as to the concentration of oil needed in the rumen and further studies are necessary to clarify this point. It appears, however, that a relatively high level of oil in the water is needed to control bloat. This probably is due at least in part to irregular consumption of oil via this route. It is also possible that the oil is removed rather rapidly from the rumen and/or is altered within the rumen. Therefore, other methods of administering the oil (such as in a concentrate mixture) where time and rate of consumption can be controlled more effectively warrant investigation. Other oils and other defoaming agents also should be examined. A number of such possibilities are being investigated.

Although the oil appeared to have some beneficial effect upon feed-lot bloat, the results were not conclusive. There was, however, a very low incidence of bloat throughout the feed-lot study in experiment I and the high proportion of zero maximum bloat values may have obscured the true effect of the treatment.

Intraruminal administration of the oil (either by injection or by stomach tube) appears to have promise as a therapeutic measure if the oil can be administered to the distressed animal in sufficient time to release the gas before the bloat reaches a fatal stage. On the other hand, it must be recognized that appraisal of the efficacy of a treatment is difficult because of the frequent recovery of severely bloated animals in the absence of any treatment. It is difficult, if not impossible to determine accurately when the bloating has progressed to the point where in the absence of treatment the animal will not recover (except perhaps in the most terminal stage where recovery even with treatment sometimes is doubtful).

The palatability of the oil appears to be high, as its addition to the water consistently increased water consumption. At no time in either of the studies was there any evidence that oil feeding lowered feed consumption or caused any other ill effects.

Average daily weight gains furnished the basis for much conjecture. Gains were higher for groups receiving the water dispersible oil; however, it is possible that the sudden removal of the oil from the water may have depressed weight gains during the subsequent control period. The low gains during control periods in phase 2, experiment I are particularly difficult to interpret. There was considerable variation in gains from period to period and occasionally weight losses or very low gains were observed,

probably due to the comparatively short duration of the experimental periods and the major effect of time of feed and water consumption on such "apparent" weight changes. The extent to which the lard oil was used as a source of energy and/or altered utilization of other nutrients was not determined in this study. Further studies designed to clarify these and related questions are in progress.

The differences in bloat incidence and severity as well as average daily weight gains varied considerably from trial to trial in experiment II; however, the trends of the two groups were similar irrespective of treatment. A large part of the differences among the periods in phase 1 probably can be accounted for by the difference in the quality of the alfalfa. At the beginning of period 1 of this phase, young lush alfalfa was fed; however, quality of alfalfa deteriorated rapidly. Bloat incidence was high initially and declined quite rapidly to the end of period 1. The large weight gain during this period probably was due to difference in fill and the fact that the animals were thin at the beginning of the experiment. During the first half of period 2 the alfalfa was more mature (1/10 bloom stage). In the second half of this period, young, lush second growth alfalfa was fed; this material was very high in moisture content and apparently the animals were unable to consume enough to produce satisfactory weight gains. Because of a lack of rainfall, high quality second growth alfalfa was not available for period 3. Therefore the animals were fed first growth alfalfa which was more mature and low in moisture content. A very low incidence of bloat and more rapid weight gains were observed.

The alfalfa fed during phase 2 was second growth forage of good quality. However, the supply was limited and the moisture content was relatively low. The differences in bloat incidence and severity and weight gains during this phase as compared to the other two phases possibly can be attributed to the high level of concentrate feeding.

The alfalfa fed during phase 3 was a very rapidly growing forage produced subsequent to late summer rains. For 16 days of this period very intense bloating occurred; on 4 days all animals in the lot showed some degree of bloat. Animals in both groups bloated severely. Several animals required treatment to prevent possible death during this phase, whereas no treatment was required during either of the other two phases. The sharp increase in bloat severity occurred simultaneously in the animals on pasture and those receiving soilage, even though the sources of feed were fields about 2 miles apart.

Analysis of data from periods when neither group of animals received penicillin indicated that one group had a greater bloating potential than the other group (even though initially the bloating potential seemed to be similar in the two groups). Since this was the group receiving penicillin throughout phases 2 and 3 of experiment II, this may account in part for the greater incidence and severity of bloat in the penicillin group. This group also served twice as the control group in phase 1; therefore,

this possible difference in bloat potential of the two groups may explain the apparent beneficial effect of penicillin in this trial. Regardless of this difference, it is evident that under the conditions of this experiment penicillin did not offer sufficient protection from bloat to justify its use as a prophylactic agent. It seems possible that penicillin may have an effect initially but has less or no bloat reducing effect after continuous feeding for several days or weeks. Additional work on the relationship of penicillin feeding to bloat incidence is needed to assess the prophylactic value of this antibiotic. Moreover, its apparent beneficial effect under some circumstances should be valuable in the future clarification of the etiology of bloat.

Summary

A water-dispersible oil markedly reduced the incidence and severity of bloat, even during periods of very severe bloating, when fed at levels of 1 and 2% in the drinking water of Holstein steers grazing alfalfa pasture. The effect was less in feed-lot bloat but incidence and severity also were less. In most instances the animals fed oil had higher average daily weight gains than controls.

Procaine penicillin (75 mg. per animal per day) appeared to reduce bloat when first administered to steers receiving alfalfa soilage. However, later in the season, when the bloat-producing potential of the forage increased sharply, animals fed penicillin had a high incidence and severity of bloat as did the controls. Penicillin feeding apparently had no effect on weight gains. Limited observations were made on the effect on bloat of various oils sprayed on fresh alfalfa soilage. Results indicated that a level of 0.25 lb. of oil per animal per day greatly reduced bloat.

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EFFECT OF PROGESTERONE ADMINISTRATION ON REPRODUCTIVE EFFICIENCY[1, 2]

K. R. JOHNSON, R. H. ROSS AND D. L. FOURT[3]

University of Idaho, Moscow, Idaho

EMBRYONIC death has been shown to be a direct cause of lowered fertility in repeat breeding cows. Tanabe and Casida (1949) reported that 66% of the repeat breeder cows with no clinical detectable abnormality had fertilized ova. At 34 days those having normal embryos had decreased to 23%. Since these ova lived for a time after fertilization, it is reasonable to believe that embryonic death may have been due to failure of the embryo to implant in the uterus. This failure could be the result of the uterus not being prepared for implantation. One of the conditions essential for the implantation of ova in the uterus appears to be a secretory condition of the endometrium and this depends largely on progesterone. Insufficient available progesterone at the time of nidation could be a major cause of "repeat breeders".

The object of this study was to determine the effects of progesterone administration on reproductive efficiency in normal cows.

Experimental Procedure

All the females in the University of Idaho dairy herd were given rectal examinations at estrus when the animal was due for first breeding. Cows 70 days post-partum, Jersey heifers 15 months, and Holstein heifers 18 months of age that had been in estrus 18–23 days previously were examined. Animals in which the ovaries were of normal size (2 to 4 cm. in diameter for cows and 1.5 to 3 cm. for heifers) with a single well-developed Graafian follicle, and free from detectable infection and disease, were considered to be ready for breeding.

The animals were bred twice during the estrus period by artificial insemination. The first service was near the middle of the estrus period and the second service 12 hr. later. The semen used was from the University herd sires. Collections were made twice weekly (Monday and Friday). The semen was scored for color and motility when collected. Semen scoring less than 9 for motility on a 1 to 10 basis was not processed for use. The semen was diluted 1 to 20 with homogenized milk heated to 92° C. for 1 minute and containing 0.1 g. of streptomycin per 100 ml. The semen

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was stored in a refrigerator at 39° F. All semen was scored for motility before each use; semen scoring less than 9 on a 1 to 10 basis was not used. No semen was used after being stored more than 4 days.

After the animals were bred, alternate cows were treated with a reposital-type progesterone while the other animals were not treated and were used as controls. The treated group received 100 mg. of a reposital progesterone intramuscularly on the 2, 3, 4, 6 and 9th day after the first breeding only. This dosage level and time interval was determined from the success of preliminary work at this station in the treatment of repeat-breeder cows. Johnson (1955) treated 20 females having had from 4 to 9 infertile services to a male of known fertility with a reposital progesterone. Sixteen of the females conceived on first breeding, three on second and one on third breeding after treatment, giving a breeding efficiency of 80.0%.

All the animals were examined for pregnancy by rectal palpation at 6 weeks, 3 months and 5 months after date of breeding. The conception data used to determine breeding efficiency in this report were taken from the 5-month palpation results.

Results and Discussion

One hundred and thirty-nine females of the Holstein and Jersey breeds were given a rectal examination at the estrus the animals were due for first breeding and were considered ready for breeding. Fifty-three of the animals were treated with a reposital-type progesterone at first service. Thirty-six conceived to first service for a breeding efficiency of 67.9%. Thirteen of the animals conceived to a second service, two to a third and two to a fourth service without further treatment. The 53 animals conceived on a total of 76 services for a breeding efficiency of 69.7%.

Seventeen additional females were given 500 mg. of 17-alpha-hydroxy-progesterone-17-n-caproate (Delalutin) intramuscularly on the second day after breeding. Thirteen of the 17 animals conceived to first service for a breeding efficiency of 76.5%. The other four animals returned to estrus on the twenty-first day, were rebred and conceived to that service.

In all cases in which the animals did not conceive after being treated with either progesterone or Delalutin, the length of the subsequent estrual cycle was not altered.

The remaining 69 animals were not treated with progesterone and were used as controls. Twenty-nine of these animals conceived to first service for a breeding efficiency of 42.0%. Twenty-six conceived to a second service, nine to a third and five to a fourth service for a total breeding efficiency of 53.9%.

The combined treated groups had a breeding efficiency of 72.2%, 70 conceptions from 97 services. The control group had a breeding efficiency of 53.9%, 69 conceptions from 128 services. Comparing the breeding efficiency from the first two services for each group, the results are 72.5% for the treated group and 50.5% for the control group.

The complete results for the two groups are shown in table 1.

The difference in breeding efficiency between the treated and control groups was significant at the .05 level by Chi-square. According to Andrews (1949) 55% or 1.8 services per conception is generally accepted as the average breeding efficiency for dairy cattle. The number of services per conception as listed by other workers are 2.02 by Bowling *et al.*, (1940), 1.7 to 2.0 by Asdell (1948), 1.8 to 2.5 by Lush (1949), and 1.72 by

TABLE 1. EFFECT OF 500 MG. PROGESTERONE FOLLOWING FIRST SERVICE UPON BREEDING EFFICIENCY OF DAIRY COWS

Items	Number of cows bred	Cows conceiving on service number—				Percent conceived on service number—				Percent breeding efficiency
		1	2	3	4	1	2	3	4	
Treated										
Holstein cows[a]	20	17	2		1	85.0	10.0		5.0	80.0
Holstein heifers[a]	9	5	3		1	55.5	33.3		11.1	60.0
Jersey cows[a]	15	10	3	2		66.7	20.0	13.3		68.2
Jersey heifers[a]	9	4	5			44.4	55.5			64.3
Cows treated with Delalutin[b]	17	13	4			76.5	23.5			81.0
Total	70	49	17	2	2	70.0	24.3	2.9	2.9	72.2
Untreated										
Holstein cows	20	13	9	1	2	52.0	36.0	4.0	8.0	59.5
Holstein heifers	12	6	2	4		50.0	16.7	33.3		54.5
Jersey cows	21	7	10	2	2	33.3	47.6	9.5	9.5	51.2
Jersey heifers	11	3	5	2	1	27.3	45.5	18.2	9.1	47.8
Total	69	29	26	9	5	42.0	37.7	13.0	7.3	53.9

[a] Received 100 mg. reposital progesterone intramuscularly on the 2, 3, 4, 6 and 9th day after the first breeding only.

[b] Received 500 mg. Delalutin intramuscularly on the second day after first breeding only.

Gilmore (1952). The expected number of services per conception used in calculating Chi-square in this report was 1.8.

Most workers agree that embryonic death is one of the major causes of reproductive failure. Laing (1949) reported 25% embryonic death in a group of 48 beef heifers that were slaughtered at 25 days post-insemination. Britton (1947) states that the early equine abortion is a relatively common cause of sterility and accounts for the majority of abortions in this species.

Experiments with laboratory animals have indicated that corpora lutea, by supplying progesterone, may be responsible for the progestational changes in the uterus which lead to nidation of the ova and pregnancy. Courrier (1935) showed that with the help of progesterone, nidation can occur in the rabbit even though the ovaries have been removed. The favoring influence of progesterone in preparing the uterus to receive and maintain the ova may be used to increase fertility. D'Amour, *et al.* (1933) gave daily doses of 50 mg. of luteal hormone to rats during the first few days after copulation. They found a much higher percentage of living embryos than is usual.

Conversely, Sammelwitz *et al.* (1956) found no effects on embryonal survival from progesterone injections in rats. These same workers injected gilts at the rate of 50, 100, 200 and 400 mg. of progesterone daily for 26 days after mating. At the time of slaughter 92% of the ova had survived in the control group, 87% in the 50 mg. group, and 64% in the 100 mg. group. In some of the sows getting 200 mg. and in all the sows getting 400 mg. the corpora lutea were completely degenerated by the time of autopsy and could not be counted. In contrast, corpora lutea of rats treated with relatively higher doses of progesterone throughout pregnancy, remained grossly histologically normal.

Warnick *et al.* (1957) injected 42 gilts with 25 mg. of progesterone every other day during the first 25 days of gestation. The progesterone treatments had no significant influence on the number of embryos present at slaughter or live pigs.

Reifenstein (1957) reported that 85 women with at least three consecutive abortions immediately preceding the pregnancy in which they were treated, were treated with 17-alpha-hydroxyprogesterone-17-n-caproate (Delalutin). In previous pregnancies, the 85 cases had a fetal salvage rate of about 14%. Under Delalutin treatment, the salvage rate increased to about 70%.

The role of progesterone in reproductive efficiency may be specie specific. In the papers reviewed the effects varied from little to no effect in rats, an adverse effect in swine, to an apparent beneficial effect in humans.

These data indicate that progesterone administered after breeding may increase the breeding efficiency of dairy cows. In this study the treated group had a first service conception of 70.0%, while the control group had 42.0%, a difference of 28.0% in favor of the treated group. The administration of progesterone may have increased the secretion of the endometrium (uterine milk), which may have aided nidation and thus reduced the incidence of embryonic death.

Summary

The effect of the administration of 500 mg. of progesterone on reproductive efficiency was studied, using 139 apparently normal cows and heifers. The animals in both the treated and control groups were free from detectable infection, disease and other reproductive abnormalities; and all of them had well developed Graafian follicles at the time of breeding. The 70 treated animals had a first service conception of 70.0%, while the 69 controls only had 42.0%. The administration of progesterone to apparently normal cows at first breeding increased the breeding efficiency of the treated group 28.0% over the controls for first service conceptions. The total breeding efficiency for the treated group was 72.2%, 70 conceptions from 97 services compared to 53.9%, 69 conceptions from 128 services for the controls.

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EFFECTS OF MEAT MEAL ON STEER PERFORMANCE AND RUMEN MICROBIAL ACTIVITY [1]

I. A. DYER [2] AND D. W. FLETCHER [2]

State College of Washington, Pullman [3]

RUMEN microorganisms play an important role in regulating rates of gain and efficiency of feed utilization by cattle. Consequently, the nutrition of the rumen microflora and fauna is of especial importance to the well-being of the host. Although many data have been collected on the function of the rumen biota (Hungate, 1950, 1956; Doetsch *et al.*, 1952) including information regarding the nutritional requirements of certain rumen bacteria (McNeill *et al.*, 1954; Bryant and Doetsch, 1955) and protozoa (Gutierrez, 1955), knowledge concerning the effects of many supplementary feeds is lacking.

Certain feeds or supplements are known to contain "growth" factor(s) for ruminants (MacLeod and Brumwell, 1954; Garner *et al.*, 1954; Ruf *et al.*, 1953; Bentley *et al.*, 1952; Hall *et al.*, 1955). The object of these experiments was to determine whether meat meal contains a supplementary growth factor for fattening cattle and to correlate the effect, if any, with rumen microbial activity as measured by short term manometric experiments.

Materials and Methods

Twenty-four steers were allotted into four groups of six steers each and individually self-fed for 96 days. Random assignments of animals to treatments and treatments to lots (1–4) were made. All steers had access to automatic waterers. Wood shavings were used as bedding. The steers were individually weighed at 28-day intervals.

Rations were compounded so that 6.75, 4.36, 2.18 and 0% of meat meal was used for groups 1 to 4 respectively Soybean oil meal replaced meat meal in groups 2 to 4 so that all rations were isonitrogenous. Ingredients of the rations other than the protein supplements and beet pulp were held constant in the various rations (table 1). All rations were pelleted into 3/16 inch cubes. Digestibility was determined by the lignin-ratio technique (Ellis *et al.*, 1946). Analysis of variance (Snedecor, 1946) was used to test for significance among treatments.

Microbial activity of the rumen contents of animals from each of the test rations was determined manometrically. Samples were obtained from randomly selected animals. These samples were removed by stomach tube

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[2] Departments of Animal Science, and Bacteriology, respectively, State College of Washington.

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approximately two hours after feeding and immediately transported to the laboratory. Ten-gram samples of rumen contents from each of the animals were weighed into duplicate 160-ml. Warburg vessels. The vessel contents were diluted with 30 ml. of a balanced salt solution with the following percentage composition: $NaHCO_3$, 0.5; $CaCl_2$, 0.01; $(NH_4)_2SO_4$, 0.05; KH_2PO_4, 0.05; K_2HPO_4, 0.05; and $MgSO_4$, 0.01; in tapwater (Hungate *et al.*, 1955).

The vessels were attached to mercury manometers, and placed in a water bath at 38.9 C. The air within the vessels and manometers was displaced with 100% carbon dioxide. Manometer readings were begun within 75 minutes after removal of the rumen contents. Experimental details are reported elsewhere (Hungate *et al.*, 1955, 1955a).

In order to increase the activity of the samples, 1.0 gm. of finely ground alfalfa hay added with the rumen contents provided an active and plentiful substrate. Since the addition of excess substrate as ground alfalfa hay would tend to stimulate one nutritional type, the cellulolytic microflora, a second substrate containing glucose, maltose, and cellobiose was also used. One ml. of a solution containing equal parts 5% glucose, maltose, and cellobiose was added from a side arm after such time as required to determine endogenous fermentation rates. In all experiments, pressure changes were recorded at five minute intervals. After 65 to 200 minutes the fermentation was stopped by the addition of 6N H_2SO_4. Methane was determined, using the method of Hungate *et al.* (1955).

Total fermentation products were determined by the use of a single vessel constant. This constant was calculated as follows:

$$0.8\ K_{CO_2} + 0.2\ K_{CH_4} = K_{\text{total products.}}$$

In contrast to earlier reported experiments (Hungate *et al.*, 1955), methane produced amounted to approximately 20% of the total products.

Results and Discussion

Meat meal in the ration at levels of 6.75 and 4.36% did not increase rates of gain and efficiency of feed utilization over the ration containing soybean oil meal as the only protein supplement. These results agree with the report of Bentley *et al.* (1952). A combination of 2.18% meat meal and 5.75% soybean oil meal resulted in an increased rate of gain which approached significance (5% level).

Rates of gain and daily feed intake were directly proportional. Efficiency of feed utilization was slightly higher in group 3 where the rates of gain were greatest. This difference was not statistically significant.

Digestibility of crude fiber and ether extract were slightly higher where soybean oil meal was used. Since such a small difference occurred among the treatments, they apparently exerted little or no effect on rates and efficiency of gain. Samples of rumen contents for the determination of

in vitro microbial activity were obtained at 7-day intervals during the last 60 days of the feeding experiment. At each sampling a careful, but subjective, evaluation of the sample was made. Samples were judged on the basis of color, relative amount of solids, and homogeniety and were rated excellent, good, fair, or poor. An excellent sample had a dark green color, a high solid content, and the consistency of a thick slurry. Samples judged poor were brown and were predominately fluid with a small amount of suspended solids.

TABLE 1. EFFECTS OF MEAT MEAL ON THE PERFORMANCE OF FATTENING STEERS

	Lot numbers and treatments			
Item	1 Basal[a] 6.75% meat meal 0% soybean oil meal	2 4.36% meat meal 2.85% soybean oil meal	3 2.18% meat meal 5.72% soybean oil meal	4 0% meat meal 8.58% soybean oil meal
No. steers on test	6	6	6	5
Av. initial weight, lb.	785	776	787	778
Av. final weight, lb.	925	925	968	940
Days on test	96	96	96	96
Av. daily gain, lb.	1.53	1.56	1.89	1.59
Av. daily feed, lb.	13.1	14.3	15.5	14.0
Av. gain per 100 lb. feed, lb.	11.1	10.9	12.2	12.1
Av. digestibility of:				
crude fiber	25.1	25.0	28.2	29.7
ether extract	92.3	93.4	92.1	94.0

[a] Composition, %: 25 sun cured alfalfa, 7 rolled oats, 7 molasses, 8.06 beet pulp, 6.75 meat meal (55%), 0.19 urea, 40 barley, 5 fat, 0.5 bone meal and 0.5 salt.

Initial experiments performed without the addition of substrate clearly showed that the observed differences in activity were due to variations in the quality sample and were not related to the presence or absence of meat meal. Samples consistently showing greater activity contained larger amounts of solids which probably represented a greater available substrate concentration. In subsequent experiments only these samples judged excellent or good were used.

Endogenous fermentation rates of rumen contents from animals on ration 3 were consistently lower than rumen contents taken from animals on the other three rations. Fermentation rates of rumen contents from animals fed rations 1 and 4 were similar and greatest, while the rate of fermentation of rumen contents from animals on ration 2 were intermediate (table 2 and figure 2). Only the differences between rations 1 and 3 and 4 and 3 were significant. This is based on results from 8 different experiments in which the difference between the means was more than twice the standard error of the difference in both cases.

The inverse relationship between those animals (lot 3) showing the greatest rate of gain and minimal fermentation rates was not expected. This result, however, could be explained on the basis of a more rapid *in vivo*

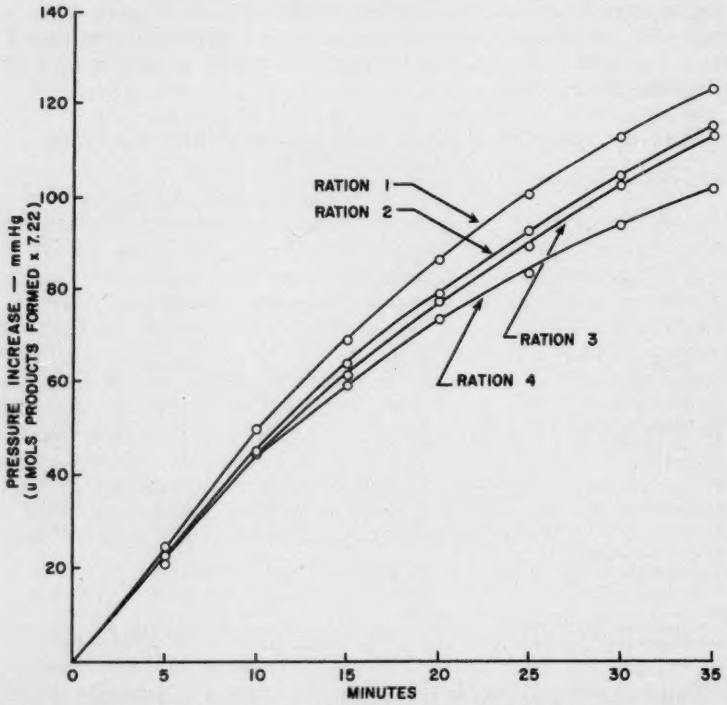

Figure 1. A comparison of the rate of fermentation of ground alfalfa hay by rumen contents obtained from animals fed rations containing the following percentage meat meal: 1(6.75), 2(4.36), 3(2.18), and 4(0).

utilization of the ingredients in the ration. Thus rumen contents deficient in available substrate at the time of sampling would result. This is indicated by the apparent increased efficiency of feed utilization by those animals in this group.

To test this hypothesis, an excess of readily available substrate was added. If the hypothesis were correct, the excess substrate should eliminate the effect. Addition of substrate as a mixture of soluble sugars however did not change the differences in the rate of fermentation (figure 2). Thus the addition of substrate as the principal hydrolysis product of the major carbohydrates in the ration yields essentially the same results as those experiments without the addition of a substrate (figure 1). It therefore

must be concluded that the ration containing 2.18% meat meal (ration 3) inhibited fermentation.

Addition of ground alfalfa hay showed that the fermentation rate increased in those samples obtained from animals in which meat meal was

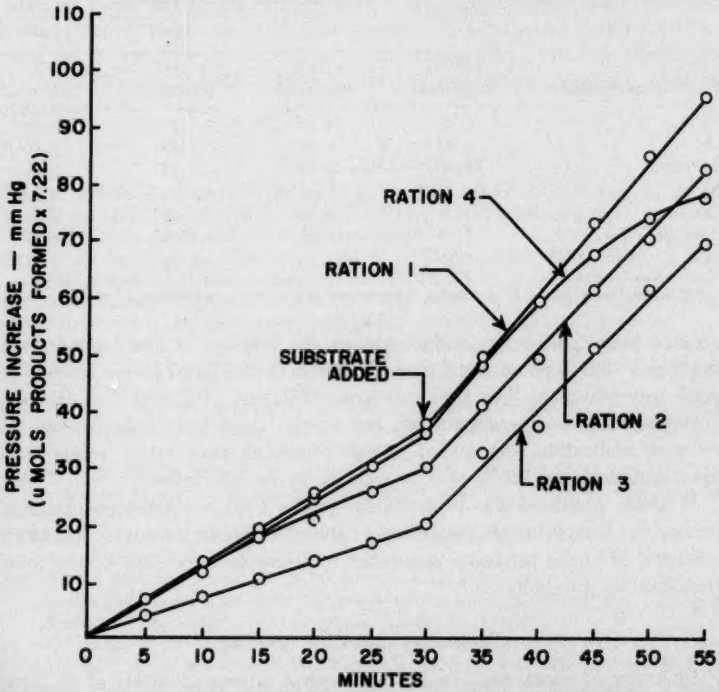

Figure 2. Comparison of the endogenous fermentation rates of the four test rations and the effect of the addition of substrate as excess soluble carbohydrate (1.0 ml. of a solution containing equal parts of 5% glucose, maltose, and cellobiose).

included in the ration. Although not proportional, the rate of fermentation in general increased with the concentration of meat meal (table 3). These results indicated that those microorganisms capable of attacking alfalfa hay were stimulated by a factor present in the ration, presumably the meat meal.

Meat meal had little effect on the performance of steers under the conditions of these tests. However, *in vitro* experiments indicate that meat meal contains a factor stimulatory to the agents of cellulose digestion. It is probable that the reason for the inability to observe this effect as in-

TABLE 2. ENDOGENOUS FERMENTATION RATES[a] OF RUMEN CONTENTS OBTAINED FROM ANIMALS FED RATIONS CONTAINING VARIOUS CONCENTRATIONS OF MEAT MEAL

	Micromols total fermentation products formed per minute Ration numbers and treatments			
	1	2	3	4
Experiment numbers	6.75% meat meal	4.36% meat meal	2.18% meat meal	0% meat meal
6a	9.06	6.70	4.15	8.64
b	8.45	6.70	4.70	9.40
Average	8.80	6.70	4.45	9.06
7a	9.75	5.39	3.25	8.64
b	9.75	4.15	2.90	7.75
Average	9.75	4.80	3.05	8.21
3a	6.50	9.40	5.80	7.25

[a] Rates determined from 0 to 30 minutes. Refer to text for manometric procedures.

creased steer performance was due to the quality of the basal ration employed. The high concentration of starch in the basal ration tended to mask any effects as increased cellulose utilization. Although the primary purpose of these experiments was not to test the effect of meat meal on cellulose utilization, the use of a high roughage basal ration might well have demonstrated an *in vivo* as well as an *in vitro* effect.

Methane produced was proportional to the total fermentation products formed in all experiments, regardless of the substrate or the source of rumen contents. Methane produced amounted to approximately 20% of the total fermentation products.

Summary and Conclusions

Inclusion of meat meal in steer fattening rations at levels of 0, 2.18, 4.36, and 6.75% had no pronounced effect upon steer performance. Effi-

TABLE 3. FERMENTATION RATES[a] OF GROUND ALFALFA HAY

	Micromols total fermentation products formed per minute Ration numbers and treatments			
	1	2	3	4
Experiment number	6.75% meat meal	4.36% meat meal	2.18% meat meal	0% meat meal
4 27.5 Min.	25.00	23.85	23.50	20.90
5 35.0 Min.	22.30	23.90	20.20	17.20

[a] Refer to text for manometric procedures.

ciency of feed utilization, digestibility of crude fiber and ether extract, and rate of gain were similar at all levels. The rate of gain and efficiency of feed utilization at a concentration of 2.18% meat meal were slightly greater but were not statistically significant. Endogenous fermentation rates, as measured by short term manometric experiments, were minimal from rumen contents obtained from animals on a ration containing 2.18% meat meal. *In vitro* fermentation of alfalfa hay was directly related to the concentration of meat meal in the ration, suggesting increased cellulose utilization.

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RELATION OF FORAGE CAROTENE TO PLASMA CAROTENE AND VITAMIN A OF RANGE COWS [1]

WARD W. REPP AND W. E. WATKINS [2]

New Mexico Agricultural Experiment Station

SEVERAL investigators have been concerned with the vitamin A nutrition of cows grazing ranges in the western United States (Hart and Guilbert, 1928; Van Arsdell *et al.*, 1950; Lewis, 1955; Wheeler *et al.*, 1957). From results obtained from an 8-year study in southern New Mexico, Watkins and Knox (1954) concluded that the carotene content of range forage was sufficient to maintain blood plasma carotene and vitamin A of breeding cows at a level meeting the animal's requirements. The investigation reported upon here was undertaken to determine whether range forage from several other locations representative of the important ranching areas of New Mexico would maintain normal blood carotene and vitamin A in range cows.

Procedure

Arrangements were made with six ranchers from representative ranching areas of New Mexico to obtain blood samples from 12 range cows each month beginning in November and continuing through May. A composite forage sample was collected from each ranch at the time the blood samples were taken. The November-through-May interval was chosen, since it represents the dormant period for most range grasses in New Mexico and the period during which vitamin A deficiency would be most likely to develop.

Collections were begun in November, 1954 and were continued for three seasons, ending in May, 1957. Approximately 1500 blood samples and 125 forage samples were collected during the 3-year study. The range cows used in this experiment varied in age from 2 to 10 years and were in various stages of pregnancy and lactation. It was not possible to make collections from the same animals each month. These cows had been grazing on forage high in carotene during the late summer and early fall and were presumed to have high liver stores of vitamin A as they went into the winter period.

[1] Journal Series No. 116, New Mexico Agricultural Experiment Station, State College, New Mexico.
[2] Assistant animal husbandman and nutrition chemist, respectively, New Mexico Agricultural Experiment Station, New Mexico College of Agriculture and Mechanic Arts.

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Figure 1. Portable equipment used in collecting blood plasma in the field.

The blood was treated with anti-coagulant, centrifuged in a battery-driven, portable centrifuge (figure 1) and the plasma refrigerated for subsequent analysis. Analysis for carotene and vitamin A was made by the method of Sobel and Snow (1947). The composite forage samples were analyzed for carotene by the method of Bickoff *et al.* (1952).

Results and Discussion

Seasonal fluctuations in plasma carotene and vitamin A and forage carotene for the three years are presented graphically in figure 2. Each point represents an average of the six ranches. It can be seen that the curves are similar in shape, indicating a close relationship between forage

TABLE 1. FORAGE CAROTENE EXPRESSED AS MILLIGRAMS PER POUND

Year	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Ranch no.	Precipitation [b] % of average Oct.–May
1954–55	4.1	0	1.0	0	0	0	2.6	1	41.3
	6.7	0	1.0	1.5	0	0	....[a]	2	119.4
	12.4	0	1.5	1.0	0	0	25.8	3	85.3
	12.4	6.0	6.7	9.3	3.6	2.1	25.8	4	101.5
	22.2	18.0	7.7	1.0	4.1	2.6	30.9	5	106.7
	7.2	0	2.1	2.6	0.5	24.7	47.4	6	70.2
1955–56	0	0	0	0	0	0	6.2	1	56.2
	0	0	0	0	0	3.6	7.2	2	41.0
	0	0	0	0	1.0	3.6	6.2	3	21.9
	6.2	5.2	6.7	11.3	9.3	12.9	7.2	4	61.7
	2.6	0	3.6	2.4	2.1	6.2	6.2	5	68.6
	5.7	5.7	6.2	0	5.2	7.2	10.3	6	28.8
1956–57	2.6	0	0	0	3.1	0	12.4	1	116.6
	0	0	0	0	1.0	0	9.3	2	103.6
	0	0	3.1	0	1.0	0	29.9	3	66.5
	3.1	0	3.1	1.0	2.1	5.2	2.6	4	57.4
	0	0	3.1	0	0	3.1	8.2	5	53.4
	0	0	0	0	5.2	10.3	24.7	6	41.1

[a] No data collected.
[b] Precipitation data, 25-year average.

carotene and plasma carotene and vitamin A. The lowest plasma values were usually recorded in February and March, with values increasing during April and May, when range plants began growing again.

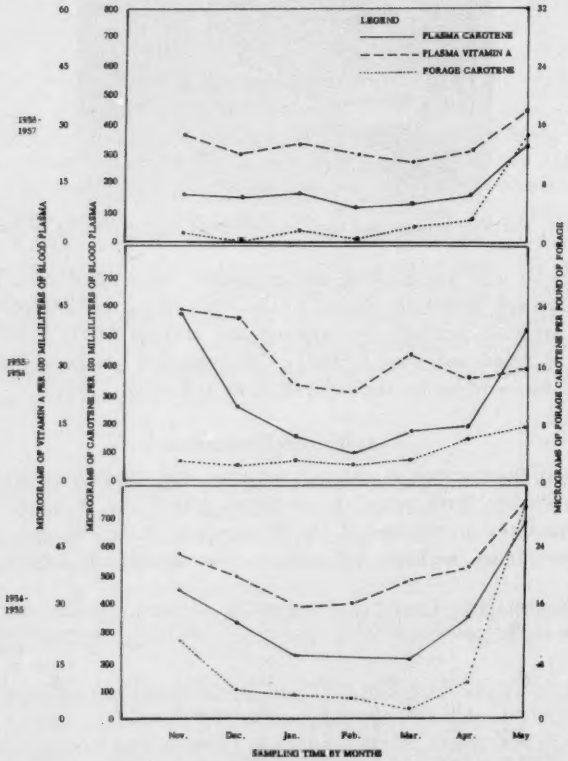

Figure 2. Carotene in range forage and carotene and vitamin A in blood plasma of range cows in New Mexico, 1954 to 1957.

A carotene analysis of the composite forage samples appears in table 1. The percentage of average October-through-May precipitation is also indicated in this table. Zero carotene values occurred in the forage for as many as 6 consecutive months and zero values for 3 or 4 consecutive months were not uncommon. The precipitation data show that average moisture conditions were considerably below normal on all ranches except ranch No. 2 for the 3-year period. During 1955–56, ranch No. 2 also was far below average. The October-through-May average was used because it

was believed that precipitation during this period would have the greatest influence on plant growth in the winter-spring period.

Figure 3 illustrates the regression of blood plasma vitamin A upon the logarithm of forage carotene intake expressed as milligrams per pound of the composite forage sample. Since the assumption was made that the forage samples analyzed for carotene content were representative of forage eaten by the cattle it is believed that mg. carotene per lb. of this forage is a valid expression of carotene intake. The correlation of plasma vitamin A with log forage carotene intake was significant at the 5% level of probability and approached significance at the 1% level of probability. This linear relationship of plasma vitamin A with carotene intake in cattle has been reported by Dolge *et al.* (1955) and Thomas and Moore (1952), the latter workers reporting that this relationship existed only until carotene intake reached a level of about four times the minimum requirement. Thomas and Moore (ibid.) wisely suggested caution in attempting to estimate plasma vitamin A levels from carotene intake. However, it is possible that regression equations may be helpful in estimating the vitamin A nutrition of cattle from knowledge of carotene intake. By applying the regression equation derived from this work to the 1.5 mg. per 100 lb. body weight intake suggested by Wheeler *et al.* (1957) as adequate to support normal gestation and lactation in range cows having high liver stores of vitamin A, a value of 24.1 ± 4.5 mcg. vitamin A per 100 ml. plasma with 95% confidence limits is obtained. This is considered within the range of normal values. A consumption of 20 lb. of the air-dry composite forage sample was assumed in this calculation.

Average data for precipitation and mean temperature were obtained from weather stations located at or near the six ranches. The multiple regression of October-through-May precipitation in inches and average monthly mean temperature upon carotene content in milligrams per pound was calculated. In calculating this multiple regression, differences between ranches and years were removed. Partial regression coefficients of 0.3009 for temperature and 1.6114 for precipitation were obtained, providing the following prediction equation:

$$\text{Carotene} = \overline{X}\ \text{carotene} + 0.3009\ (X_{temp} - \overline{X}_{temp}) + 1.6114\ (X_{prec} - \overline{X}_{prec})$$

where $\overline{X}$ carotene $= 4.5$ mg. per lb.; $\overline{X}_{temp} = 47.1°$ Fahrenheit, and $\overline{X}_{prec} = 0.57$ inches.

Average monthly mean temperature varied from 23° to 77° F. Monthly precipitation ranged from 0 to 4.44 in. among the six ranches for the October-through-May period during the 3 years. Changes of 0.3009 and 1.6114 mg. carotene per pound of forage were associated with changes of one degree Fahrenheit and one inch of precipitation, respectively.

The standard partial regression coefficients were 0.1802 for precipitation and 0.4395 for temperature, indicating that temperature was about 2.5 times as important in determining carotene content of the forage as was

precipitation during the November-through-May period. The partial regression coefficient for temperature was significant at the 1% level and the partial regression coefficient for precipitation was significant at the 5% level of probability. An analysis of variance of the data revealed that the multiple regression was highly significant. The multiple correlation coefficient, $R = 0.5191$, indicated that the two variables, temperature and precipitation, accounted for about 27% of the fluctuation in forage carotene content. It is possible that, if daily precipitation and temperature data had been used and provision for delayed effect upon plant growth had been made, a larger percentage of the carotene fluctuation might have been accounted for.

An analysis of the data where variation between ranches and years was not removed did not alter the regression equation greatly. Partial regression coefficients of 0.3567 for temperature and 2.4544 for precipitation were obtained. The partial regression coefficient for precipitation was significant at the 1% level of probability whereas in the previous analysis it had been significant at the 5% level of probability. Standard partial regression coefficients were 0.2501 for precipitation and 0.5248 for temperature indicating a slight relative increase in the contribution of precipitation to carotene content when difference between ranches and years were removed. According to this analysis temperature was approximately twice as important an influence in determining carotene content of the grass as was precipitation.

Ranches 1, 2, and 3 are located in northern New Mexico. It can be seen from table 1 that there were many more months in which no carotene was found in the forage from the northern ranches than the southern. At no time during the study did the blood plasma carotene and vitamin A from cows from southern New Mexico fall below levels considered normal. These findings are in agreement with a previous investigation made in southern New Mexico (Watkins and Knox, 1954). In northern New Mexico during February and March, plasma carotene levels as low as 25 mcg. per 100 ml. and vitamin A levels of 13 mcg. per 100 ml. were recorded. Levels of this order were reported to be critical by Davis and Madsen (1941). However, Van Arsdell *et al.* (1950) and Baker *et al.* (1954) have reported that levels this low and lower, at least for short periods, were not associated with deficiency symptoms. No cows or calves from cows in this experiment exhibited visual clinical symptoms of vitamin A deficiency. Calving percentages on the two ranches where the lowest plasma carotene and vitamin A levels were recorded exceeded 90% for the three years.

In spite of attempts to gather composite samples of the forage which the cattle were actually eating, it remained difficult to assess the relative contribution of browse plants to wintertime carotene nutrition. Analysis of such browse plants as yucca and prickly pear fruit showed appreciable carotene content during winter and early spring. Even so, a significant

correlation did exist between the carotene of the composite forage samples and plasma vitamin A as indicated in figure 3. It may be that the contribution of browse plants to the carotene nutrition of range cattle operates as a factor of safety during the winter period.

Since no vitamin A deficiencies were noted and calving percentages remained high even on the two ranches where temporarily low plasma carotene

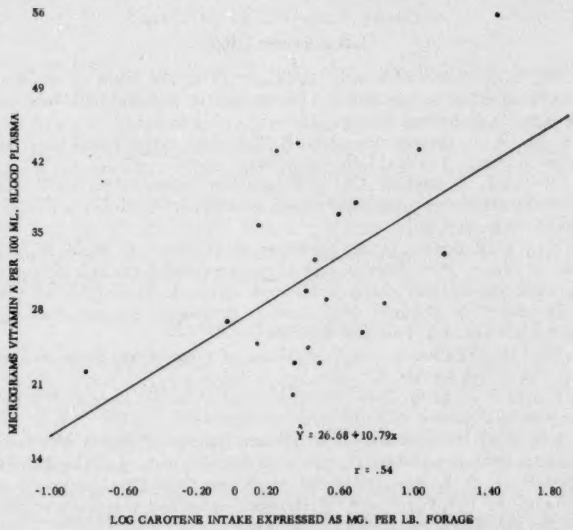

Figure 3. Regression of blood plasma Vitamin A upon the logarithm of forage carotene intake.

and vitamin A values were recorded and as this investigation was conducted during a period of extreme drought, it appears that vitamin A supplements should not be needed to maintain reproductive performance of New Mexico range cows under any but exceptional circumstances. These findings are in essential agreement with Wheeler *et al.* (1957), Lewis (1955), and Van Arsdell *et al.* (1950).

Summary

Results of a 3-year investigation of the relationship between forage carotene and plasma carotene and vitamin A in New Mexico range cows during winter and early spring are presented. A significant linear relationship was found to exist between plasma vitamin A and the logarithm of forage carotene intake. The regression of precipitation and average mean air temperature upon forage carotene was highly significant. Standard partial regression coefficients indicated that temperature contributed con-

siderably more to the fluctuation in forage carotene than did precipitation during this period. No visual clinical symptoms of vitamin A deficiency were observed in the cows used in this experiment, nor did blood plasma carotene or vitamin A drop below normal levels except for short periods. Range forage samples from southern New Mexico contained carotene during most of the months sampled. Forage samples collected in northern New Mexico contained no carotene for considerable periods.

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INTERRELATIONS OF NUMBER, AVERAGE WEIGHT AND PROGESTERONE CONTENT OF CORPORA LUTEA IN SWINE [1]

ROBERT G. LOY, W. H. MCSHAN, H. L. SELF AND L. E. CASIDA
University of Wisconsin, Madison

DIFFERENCES in the level of endocrine activity of the corpus luteum may underlie certain variations in the physiology of the estrual cycle and of pregnancy. Illustrative of the theories on problems involving such variations is the proposal of Pomeroy (1955) that the relatively greater embryonic mortality in sows with higher ovulation rates (Perry, 1954) may be due in part to an excess of progesterone. Relationships considered in the present study are those among number of corpora lutea, average weight of corpora lutea, total luteal tissue, progesterone concentration and total progesterone content of luteal tissue in gilts.

Materials and Methods

Animals from which data were obtained were predominantly of the Chester White and Poland China breeds. They were maintained on various sequences of feeding management (none involving severe restrictions of feed) and were slaughtered on the 25th, 70th and 105th days of gestation.

The corpora lutea were dissected from the ovaries at the time of slaughter and were counted and weighed by ovaries. Chemical analysis for progesterone content was done on corpora lutea of the group slaughtered at the 70-day stage of gestation only. The method of analysis has been reported elsewhere (Loy, McShan and Casida, 1957).

The data thus obtained were examined primarily by use of correlations and standard partial regressions. All correlations and regressions were calculated within breed-feed groups.

Results

The number of corpora lutea per gilt ranged from 6 to 21 and the average weight of the corpora lutea per gilt ranged from 304 mg. to 760 mg. The range of total weight of luteal tissue was from 2960 mg. to 8919 mg. The values for progesterone concentration ranged from 20 to 91 mcg. per gm. of tissue and are comparable to the range of 36 to 76 mcg. per gm. of tissue found from bioassay by Allen (1930) and the 50 mcg. per gm. of tissue in sows pregnant 40 to 75 days determined similarly by Kimura and Cornwell (1938). The progesterone content of all of the corpora lutea in a gilt ranged from 110 to 636 mcg.

[1] Published with the approval of the Director of the Agricultural Experiment Station. Paper from the Department of Genetics No. 687, Departments of Zoology and Animal Husbandry.

TABLE 1. INTERRELATIONS OF NUMBERS OF CORPORA LUTEA, AVERAGE WEIGHT OF CORPORA LEUTEA AND TOTAL LUTEAL TISSUE

Stage of pregnancy	Correlations calculated between: Ovaries within gilts: No. and av. wt. of C.L. r	d.f.	Gilts within groups: No. and av. wt. of C.L. r	d.f.	No. of C.L. and total luteal tissue r	d.f.	Av. wt. C.L. and total luteal tissue r	d.f.	No. of C.L. right ovary and no. of C.L. left ovary r	d.f.	Av. wt. of C.L. right ovary and av. wt. of C.L. left ovary r	d.f.
25 day	−0.30	25	−0.22	39	0.61**	39	0.62**	39	−0.46*	21	0.63**	21
70 day	0.40	22	−0.32	14	0.69**	14	0.44	14	−0.26	16	0.88**	16
105 day	−0.02	49	−0.43**	38	0.67**	38	0.38*	38	−0.52**	38	0.82**	38
Pooled	−0.04	98	−0.34**	93	0.66**	93	0.47**	93	−0.44**	77	0.79**	77

* $P<0.05$.
** $P<0.01$.

Correlations were calculated between gilts for the following: (1) Average weight of corpora lutea and number of corpora lutea, (2) average weight of corpora lutea and total luteal tissue, and (3) number of corpora lutea and total luteal tissue. This was done for each of the stages of gestation and also the sums of squares and cross products within stages were pooled for a within-stage correlation (table 1).

The negative correlation between number and average weight of corpora lutea (pooled, —0.34; $P<0.01$) suggests an extra-ovarian factor affecting formation and/or support of the corpora lutea which is limited in amount. Consequently, the larger the number of corpora lutea the less on the average there is of this factor to affect each of them.

TABLE 2. STANDARD PARTIAL REGRESSIONS INVOLVING TOTAL WEIGHT OF CORPORA LUTEA, PROGESTERONE CONCENTRATION, AND PROGESTERONE CONTENT

	Standard partial regression on each of two independent variables				Correlation between independent variables
Dependent variable	One independent variable	b′	Other independent variable	b′	
Total wt. C.L.	No. C.L.	0.93**	Av. wt. C.L.	0.78**	—0.34**
Prog. conc.	No. C.L.	—0.30	Av. wt. C.L.	0.34	—0.32
Prog. content	No. C.L.	0.09	Av. wt. C.L.	0.61*	—0.32
Prog. content	Total wt. C.L.	0.41	Prog. conc.	0.90**	0.00

* $P<0.05$.
** $P<0.01$.

Standard partial regressions of the total tissue on number of corpora lutea and on average weight of corpora lutea all on the linear scale (table 2) show that number is slightly but not significantly more important than average weight in determination of total luteal tissue (b′ of 0.93 as compared to 0.78).

Correlations between number of corpora lutea in the right ovary and in the left ovary, and between average weight of corpora lutea in the right ovary and in the left ovary were calculated for each of the stages of gestation and for all stages pooled (table 1).

A significant negative correlation between number of corpora lutea in the right ovary and in the left ovary (—0.44; $P<0.01$) is in accord with the concept that follicular development and ovulation occur under the influence of extra-ovarian factors which are available in limited amount also. More follicles in one ovary than in the other may be competent to utilize these factors, either through chance or because of local environment; the final result, however, is that there is less available for follicles in the opposite ovary.

The correlations between average weights of corpora lutea in the right ovary and in the left ovary (0.79, $P<0.01$), and between progesterone concentration in the right ovary and in the left ovary (0.78, $P<0.01$), suggest that factors which influence these characteristics are functioning in the animal as a whole rather than locally within an ovary.

The apparent lack of association between number of corpora lutea in an ovary and average weight of corpora lutea in the same ovary (table 1; r = —0.04) also is consistent with the principle that factors which affect corpora lutea weight are of a systemic rather than local nature. Further, the failure to find significant associations either between number of corpora lutea in an ovary and progesterone concentration in the same ovary (r = —0.03; table 3, footnote) or between average weight of corpora lutea in an ovary and progesterone concentration in the same ovary (r = 0.01; table 3 footnote) are consistent with a systemic mechanism affecting both corpora lutea weight and progesterone concentration. The multiple correlation (R = 0.004) of progesterone concentration with number and average weight of corpora lutea in the same ovary also fails to demonstrate a local influence upon progesterone concentration.

TABLE 3. RELATION BETWEEN PROGESTERONE CONCENTRATION AND CONTENT, AND THEIR RELATION TO NUMBER AND AVERAGE WEIGHT OF CORPORA LUTEA, AND TOTAL LUTEAL TISSUE [a]

Variable	No. C.L.	Av. wt. C.L.	Total luteal tissues	Progesterone content
Progesterone concentration	—0.41 [b]	0.44 [c]	0.00	0.90**
Progesterone content	—0.10	0.58*	0.41	....

[a] Correlations in body of table are calculated between gilts and based on 14 degrees of freedom.
[b] Corresponding correlation between ovaries within gilts, —0.03.
[c] Corresponding correlation between ovaries within gilts, 0.01.
* P<0.05.
** P<0.01.

Correlations involving progesterone concentration and content (table 3), and those used in calculating standard partial regressions (table 2) involving these factors, were calculated using data obtained from gilts of the 70-day stage of gestation.

Negative associations of comparable magnitudes between number of corpora lutea and average weight (r=—0.32) and between number of corpora lutea and progesterone concentration (r = —0.41, df = 14, P = 0.09), as well as a positive correlation between average weight and progesterone concentration (r = 0.44, df = 14, P = 0.09) also are in accord with a regulatory influence outside the ovary, which acts upon the number of corpora lutea present to produce a limited total weight of luteal tissue.

The apparent lack of association between number of corpora lutea and total progesterone content of luteal tissue (table 3; r = —0.10) fails to support the proposal of Pomeroy (1955) that the relatively greater embryonic mortality in sows with higher ovulation rates may be due in part to an excess of progesterone. The standard partial regression of progresterone content on number of corpora lutea when average weight of corpora lutea is held constant (table 2; b′ = 0.09) also fails to show

that number has a significant part in the determination of total progesterone content of corpora lutea.

Total progesterone in the corpora lutea of a gilt is determined by amount of luteal tissue and the progesterone concentration of that tissue. These two factors differ in their importance as a source of variability in total progesterone, all on the linear scale. The standard partial regression on concentration was 0.90 whereas on total tissue it was 0.41 ($P<0.01$ for the difference between the two regressions).

Summary

Gilts were slaughtered at 25, 70 and 105 days of gestation and numbers and weights of corpora lutea determined. Chemical analyses of progesterone were made on the corpora lutea from the 70-day stage. The correlation of number and average weight of corpora lutea was —0.34, $P<0.01$, as calculated between gilts, and —0.04 between ovaries within gilts. The correlation of average weight in the right ovary and in the left ovary was 0.79, $P<0.01$, and of number of corpora lutea in the right ovary and in the left ovary was —0.44, $P<0.01$. The standard partial regression of total luteal tissue on average weight of corpora lutea on the linear scale was 0.78, and on number was 0.93. Correlations of 0.44, $P = 0.09$, were calculated between gilts for average weight of corpora lutea and progesterone concentration, and of —0.41, $P = 0.09$, between number and concentration. Between ovaries within gilts these correlations were 0.01 and —0.03, respectively. The correlation of progesterone concentration in the right ovary and in the left ovary was 0.78, $P<0.01$. Standard partial regressions of total amount of progesterone on total luteal tissue on the linear scale was 0.41 and on progesterone concentration 0.90. A correlation of 0.00 was found between amount of luteal tissue and progesterone concentration.

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THE VITAMIN D CONTENT OF ROUGHAGES

G. C. WALLIS,[1] G. H. KENNEDY[2] AND ROY H. FISHMAN,[1]
with the cooperation of
Agricultural Experiment Stations of Florida, Illinois, Kansas, Michigan, New York, North Carolina, South Dakota, Texas, and Washington, and The California State Polytechnic College

MANY investigators have demonstrated that farm animals have a fundamental need for vitamin D. Roughages are the only natural feeds which supply significant amounts of this vitamin for livestock. Hence, quantitative information about the vitamin D content of roughages is of obvious importance. Despite the importance of roughages in livestock feeding only a comparatively few values for vitamin D content have been published. Johnson and Palmer (1941), Wallis (1944), Bechdel *et al.* (1936), Moore *et al.* (1948), and Newlander (1948) have provided some information but this pertains mostly to alfalfa.

Keener (1954) found that the vitamin D activity of fresh green plants was variable but of a low order. Ultraviolet irradiation of artificially dried samples showed that first cutting samples were potentially much poorer sources of vitamin D than subsequent cuttings made at similar stages of maturity. Weits (1954) has shown that roughages contain an antivitamin D factor.

The study reported here was designed to extend our knowledge about the vitamin D content of roughages available for livestock feeding by (1) obtaining samples from widely different regions of the United States, (2) by including a wide variety of roughages upon which no published information is available, and (3) by including a substantial number of samples of sun-cured hay. A rather wide-spread feeling exists that if hay is sun-cured it will be high in vitamin D, but published experimental evidence on this point is extremely meager.

Procedure

Ten agricultural colleges and experiment stations, widely scattered geographically, supplied samples of the types of roughages most prevalent or of particular interest in their respective localities. The samples covered a wide range with respect to quality; some were of the best, some fair, and others of poor quality. Final selection and preparation of the samples were left to the discretion of the cooperating colleges and experiment sta-

[1] Standard Brands Incorporated, New York, N. Y.
[2] 329 Orchard Road, Newark, Delaware; present address, Statistics Branch, AS Division, Fort Detrick, Frederick, Md.

tions. In all, 65 samples of roughages were studied, including 40 of sun-cured hay, 6 of mow-cured hay, 7 of winter range grass, 2 of fodder, 4 of silage, and 6 samples of dehydrated roughages. A representative sample of each roughage was obtained. A 250–350 gm. portion of each was finely ground and sent for vitamin D assay to the Laboratory of Industrial Hygiene, an independent commercial and research laboratory. For the hay samples, another portion (unground) was sent to the U.S.D.A. for government classification and grade.

TABLE 1. THE VITAMIN D CONTENT OF SUN-CURED HAY

Forage sample[a]	U.S. grade	U.S.P. units of Vitamin D per lb.[b] (air-dry basis)	Forage sample[a]	U.S. grade	U.S.P. units of Vitamin D per lb.[b] (air-dry basis)
Alfalfa			Prarie (cont'd)		
K-2	1	400	SD-5018	Sample	550
W-1 (2)[b]	1	280	SD-5019	Ungraded	510
SD-5020 (2)	1	200	Lespedeza		
SD-5021	2	690	NC-2	2	1020
			Timothy and alfalfa		
T-El Paso	2	460	NY-702 (4)[b]	1	690
W-2	2	1250	NY-777	1	910
W-4	2	830	NY-714 (3)	3	470
K-1	3	510	NY-726 (3)	Sample	900
T-Brazos	3	900	Timothy and clover		
C-SB6 (2)	Ungraded	250	NY-729	1	1110
C-4 (2)	Ungraded	80	NY-795	1	1160
C-3 (2)	Ungraded	220	NY-773	3	970
Clover			Oat and vetch		
NY-783	1	810	C-1	Ungraded	230
NY-812	2	1160	C-2	Ungraded	70
NY-799	2	1060	Brome-quack-alfalfa		
Timothy			M-4[c]	1	790
NY-802	1	1180	Alfalfa-heavy grass		
NY-709	3	1200	Mixed hay		
NY-801	3	930	M-1	2	730
NY-764	Sample	1140	M-3	Sample	1280
Soybean			Brome grass and alfalfa		
NC-3[c]	1	260	M-2	2	1140
NC-4[c]	1	700	Mixed hay		
NC-5	1	260	(grass, clover, alfalfa)		
I-1[c]	Ungraded	160	W-3	2	1210
I-2[c]	Ungraded	330	Mixed grass (redtop, clover, timothy)		
Peanut			W-5	3	1440
NC-1	2	1420	Grass hay (Brome, blue grass, alfalfa)		
Prairie			M-5[c]	Sample	570
T-Blackland	2	650			
SD-5017	3	580			

[a] Letters indicate state from which sample was obtained: C-California; F-Florida; I-Illinois; K-Kansas; M-Michigan; NY-New York; NC-North Carolina; SD-South Dakota; T-Texas; W-Washington.

[b] Multipliers and divisors for estimating the upper and lower probable limits of potency within which values would be expected to fall two out of three times in subsequent assays.

Number of assays on sample	Multiplier or divisor
1	1.315
2	1.213
3	1.172
4	1.148

Potencies were determined by single assays except as noted by the numbers in parentheses following sample designation.

All vitamin D assays were made under the direction of Miss Grace McGuire at the Laboratory of Industrial Hygiene, New York City.

[c] Mow-cured.

Estimations of the vitamin D content of each hay or roughage were made according to the Method of Assay for vitamin D essentially as prescribed in the U. S. Pharmacopoeia, Revision XIII. Statistical procedures and computations followed were those proposed by C. I. Bliss (1952). Estimates of the error to be attached to the values for potency reported in tables 1 and 2 were based upon the results of those assays conducted more than once on the same sample; i.e., the "repeatability" of the assays. (See footnote (b), table 1, for the multipliers and divisors for estimating repeatability).

Results and Discussion

The vitamin D potencies of the 40 samples of sun-cured hay and the 6 samples of mow-cured hay are shown in table 1. Samples of alfalfa, clover, timothy, soybean, peanut, prairie, and lespedeza hay are included as

TABLE 2. THE VITAMIN D CONTENT OF MISCELLANEOUS ROUGHAGES

Kind	Sample[a]	U.S.P. units of vitamin D per lb.[b]
Winter range (air-dry basis)		
Native grass	SD–5005	290
Bermuda grass		
(just after first frost–Jan.)	F–12	890
(same weathered on range–Mar.)	F–10A	290
(new growth-simultaneous with weathered sample–Mar.)	F–10B	270
Pangola grass		
(just after first frost–Jan.)	F–27(2)[b]	390
(same weathered on range–Mar.)	F–14A	350
(new growth-simultaneous with weathered sample–Mar.)	F–14B	470
Fodder (air-dry basis)		
Atlas sorgo	K–3	250
Sorghum	SD–5039	200
Silage (wet basis-as fed)		
Corn	SD–5022	80
Corn and sorghum	SD–5023	70
Hegari	T–hegari	110
Johnson grass and cane	T	90
Dehydrated roughages		
Alfalfa	K–4	280
17% alfalfa meal	C–SB3	80
17% alfalfa meal	C–SB4	110
20% alfalfa meal	C–SB5	150
Cereal grass	W–SB1	210
Cereal grass	W–SB2	170

[a] See footnote (a) table 1.
[b] See footnote (b) table 1.

well as several different kinds of mixed hay listed according to government classification and grade. The results obtained for samples of winter range grasses, fodder, silage, and artificially dehydrated roughages are shown in table 2.

For sun-cured hay, the lowest vitamin D value found was 70 U.S.P. units of vitamin D per pound in a sample of oat and vetch hay, while the highest value found was 1440 U.S.P. units in a sample of mixed grass (redtop, clover, timothy) hay. Within the limits of one kind of hay, alfalfa for instance, the range for sun-cured samples was from 80 to 1250 units per pound—a 16-fold variation. The data in table 1 show the variations found for the other kinds of sun-cured hay covered by the survey.

Samples of sun-cured hay from regions where curing conditions are generally good and sunshine abundant present some surprising and unexpected information, as for instance the values of 80, 220, and 250, respectively, for 3 samples of sun-cured alfalfa from California (see alfalfa samples, C-SB6, C-4, and C-3, table 1). The results are even more striking when compared with the vitamin D content found for dehydrated alfalfa. As shown in table 2, dehydrated alfalfa ranged from 80 to 280 units per pound which is essentially the same as for the California sun-cured alfalfa. Thus, some artificially dehydrated alfalfa was found to contain more vitamin D than some of the sun-cured samples.

These results provide substantial evidence that sun-cured hay, regardless of kind or government grade, varies over a wide range in vitamin D content. This observation is of significance because it is the vitamin D content of a specific batch of hay or other roughage, with which the livestock raiser or research man is concerned in livestock feeding. In view of the wide variations which occur, the use of an average value for the vitamin D content of a roughage may be decidedly misleading.

The mow-cured hay samples assayed from 160 to 790 units per pound which is within the range of 70 to 1440 covered by the sun-cured samples. Thus the simple knowledge that a sample of hay is mow-cured or sun-cured really indicates little or nothing with regard to its vitamin D content.

The samples of winter range grass included in this study varied from 270 to 890 units when calculated on the air-dry basis as shown in table 2. It is interesting to note that old-crop weathered Bermuda grass taken from a Florida range the last of March had a potency of 290 units while the fresh new growth taken at the same time contained 270 units on a comparable air-dry basis. Similar figures for Pangola grass were 350 and 470 units respectively. The close similarity in vitamin D content between the old weathered grass and the fresh new grass is contrary to usual assumptions.

Sorghum fodder ran fairly low, varying from 200 to 250 units per pound; silages on the wet basis as fed, 70 to 110 units; and dehydrated roughages 80 to 280 units.

The results of this study are in general agreement with the earlier observations of Wallis (1944) who stated, ". . . there are influences other than

the amount of sunshine received which greatly affect the vitamin D content of the resulting hay". They also extend the work of Newlander (1948), Moore *et al.* (1948) and Keener (1954) with reference to the variability of the vitamin D content of roughages.

Calculations from Newlander's data show an average of 216 units of vitamin D per pound in a 1946 crop of field-cured mixed timothy, grass, alfalfa and clover hay. The 1947 crop from the same field which received approximately the same curing and handling contained 862 units—approximately 4 times as much.

Moore *et al.* (1948) harvested alfalfa from the same field for silage, barn-dried hay and field-cured hay. When converted to units per pound of air-dried forage, the results for the 1946 crop show 395 units of vitamin D per pound as wilted for silage. When ready for barn-drying it had received about twice as much solar radiation but contained only 263 units per pound. As field-cured hay it had received approximately six times as much solar radiation as the wilted silage. It then contained 400 units per pound—almost the same as for the wilted silage.

To study possible effects of stage of maturity on vitamin D content of forage plants, Keener (1954) cut and artificially dried samples of ladino clover, red clover, and timothy at three stages of maturity. The average vitamin D content for the three samples (one of each forage) cut June 25 was 20 units per pound; August 2, 302 units; and September 15, 650 units. In samples of alfalfa harvested from the same field and dried inside on a barn drier, Thomas *et al.* (1951) found 96 units per pound when harvested at the bud stage, 145 units at half-bloom or hay stage, and 291 units at seed stage. Differences from year to year and from field to field appear to be much greater than differences due to stage of maturity within the limits compatible with the making of a good-quality nutritious hay. As Keener (1954) points out, "The higher vitamin D content of more mature forage is of considerable scientific interest but of little apparent practical importance because of the lowered feeding value in other respects".

Some of the variation in the vitamin D content of roughages may be related to the amount of dry, dead, and brown material in the forage as cut. Thomas *et al.* (1951) reported 2,700 I.U. of vitamin D per pound for dead leaves on the alfalfa plant, 295 units for half dead or yellowed leaves, and less than 33 units for entirely green leaves. However, Newlander (1948) reported much smaller differences. His values were 0.84 units per gram (380 units per pound) for hand picked green leaves and 1.10 units per gram (500 units per pound) for brown leaves.

Keener (1954) also reported differences in the vitamin D content of first, second, and third cuttings of ladino clover, red clover, and timothy made at comparable stages of maturity and dried artificially. First cutting samples averaged 20 units per pound; second cuttings, 151 units; and third cuttings, 216 units.

The vitamin D values reported in this study and in other scientific

literature could be influenced in varying degrees by the antivitamin D factor which has been found in roughages, Weits (1954). The evidence indicates that this factor is carotene. It measurably decreases healing responses of rachitic rats to known amounts of vitamin D.

Vitamin D values reported for artificially dried grass and for most hay samples after chromatography to remove the antivitamin D factor are higher than those made before chromatography. However, vitamin D values for roughages as made in this and other studies by methods which reflect the net vitamin D activity for the animal (true potency minus antivitamin D effects) are undoubtedly preferable and serve a more useful purpose in most situations where such information is employed.

It seems evident from the results of the present study and from the work of other investigators that the amount of sunshine received does not give a reliable indication of the vitamin D content of roughages. The fact that a hay is sun-cured and of good quality gives no assurance that it will rank high as a roughage source of vitamin D.

Summary

The vitamin D content has been determined in 65 roughage samples including sun-cured hay, mow-cured hay, winter range grass, fodder, silage and artificially dehydrated roughages. The results show that wide and unpredictable variations occur in the vitamin D content of roughages.

Sun-cured hay varied from 70 to 1440 units per pound; mow-cured hay, 160 to 790; winter range grass, 270 to 890; fodder 200 to 250; silage (wet basis) 70 to 110; and artificially dehydrated roughages 80 to 280 units per pound.

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THE INFLUX OF LEUKOCYTES AND PRESENCE OF BACTERICIDAL SUBSTANCES IN INOCULATED UTERI OF ESTROUS AND PSEUDOPREGNANT RABBITS

H. W. Hawk [1]

Dairy Cattle Research Branch, A.R.S., U.S.D.A., Beltsville, Md.

THE uteri of follicular-phase and luteal-phase animals differ in their inflammatory response to infectious agents (Black *et al.*, 1951, 1953, 1953a; McDonald *et al.*, 1952; Rowson *et al.*, 1953). The response of luteal-phase rabbit and cow uteri to inoculation with bacteria or bacterially-contaminated semen is characterized one or two days post-inoculation by massive leukocytic infiltration of the endometrium and accumulation of pus in the lumen. Inoculated uteri of estrous animals exhibit relatively little gross evidence of the infection and the endometrium is infiltrated with only moderately increased numbers of leukocytes.

The greater inflammatory response in luteal-phase animals does not reflect a more effective defense mechanism. On the contrary, large numbers of bacteria have been recovered from the uterine lumena of luteal-phase rabbits and cows at various intervals between 4 and 48 hours after inoculation, while the uteri of follicular-phase animals contained relatively few of the introduced organisms (Rowson *et al.*, 1953; Black *et al.*, 1954; Hawk *et al.*, 1955, 1957).

The above results of experimental uterine inoculation are typical of all groups of animals investigated with the exception of repeat-breeder cows. The uteri of clinically-normal luteal-phase repeat-breeders show less inflammatory response and greater bactericidal properties after experimental inoculation than do the uteri of luteal-phase maiden heifers (Black *et al.*, 1953, 1954).

The difference in numbers of bacteria which can be recovered from inoculated uteri of follicular-phase and luteal-phase animals is apparently due to greater bactericidal activity in the uteri of estrous animals, since the difference could not be attributed to differences in the nutritive properties of uterine secretions of estrous and pseudopregnant rabbits (Hawk *et al.*, 1957). The differential inflammatory response and bactericidal activity is due to the action of progesterone (Black *et al.*, 1953a, 1954; Rowson *et al.*, 1953); the hormone apparently inhibits the uterine defense mechanism of luteal-phase animals. This inhibitory action seems to be restricted to the uterus rather than being a body-wide phenomena (Hawk *et al.*, 1955).

[1] The author is indebted to J. F. Sykes for counsel in the conduct of the study and preparation of the report, and to R. D. Gaalaas for technical assistance during the course of the study.

The relative bactericidal activity of uteri in estrous and pseudopregnant rabbits can be reversed; the uterine defense mechanism of pseudopregnant rabbits was made even more effective than that of estrous rabbits by sensitization of pseudopregnant rabbit uteri with killed bacteria prior to inoculation with live ones (Hawk *et al.*, 1957). The presence of large numbers of leukocytes in the sensitized pseudopregnant rabbit uteri at the time of inoculation with live bacteria may have been responsible for this reversal; however, the role of leukocytes in the uterine defense mechanism is somewhat equivocal. The present study was undertaken to obtain further information on factors involved in the uterine defense mechanism. The first experiment was conducted to determine the relationship between leukocytic migration into the uterine lumen and bactericidal activity of the uterus during the early stages of the inflammatory response in estrous and pseudopregnant rabbits.

Experimental Animals

The rabbits used in these experiments were sexually-mature Dutch females 8 to 12 months of age. They were kept in individual cages for at least a month before use to minimize the chance of uncontrolled pseudopregnancy. Experimental ovulation and ensuing pseudopregnany were induced by mating estrous females to vasectomized bucks or by intravenous injection of 100 I.U. of chorionic gonadotrophin. Pseudopregnant rabbits were used 8, 9 or 10 days after the induction of ovulation. Estrous rabbits were in heat the day of use.

Experiment 1. Relationship between Leukocytic Response and Bactericidal Activity

Methods. Estrous and pseudopregnant rabbits were anesthetized, laparotomized and one uterine horn of each rabbit ligated at each end to prevent escape of the bacterial inoculum. The anterior end of the uterus was ligated at its point of juncture with the oviduct, care being taken not to include veins in the ligature. The cervix was ligated through an incision in the anterior vagina. The ligated uterine horn was then inoculated with 0.2 ml. of a saline suspension of a 24-hour culture of *Escherichia coli* by puncture of the anterior uterine wall with a 26 gauge hypodermic needle. The needle was passed through a part of the posterior cervix to prevent leakage of the inoculum through the puncture. Two or four hours after inoculation, the rabbits were killed, the uterus removed and the inoculated horn of each rabbit flushed as aseptically as possible with 10 ml. of sterile physiological saline. The washings from each uterine horn were diluted serially and duplicate 1 ml. aliquots of appropriate dilutions were mixed with violet red bile agar. The plates were incubated at 37° C. for 24 hours, the colonies counted and the total number of *Esch. coli* recovered from each uterine horn was calculated.

The number of leukocytes recovered from each uterine horn was estimated by microscopic examination of the original uterine flushings or the first 10-fold dilution. Several 0.01 ml. aliquots of the well-mixed flushings were spread evenly over a circular area 1 cm. in diameter, dried and stained with toluidine blue. Each stained circle of uterine debris was sampled by microscopic sweeps along 2 diameters at right angles, the leukocytes were counted and the total number flushed from each uterine horn was calculated.

TABLE 1. LOG NUMBERS OF *ESCHERICHIA COLI* AND LEUKOCYTES RECOVERED FROM THE UTERI OF ESTROUS AND PSEUDOPREGNANT RABBITS 2 OR 4 HOURS AFTER UTERINE INOCULATION [a]

In utero incubation time (hr.).	Type of rabbit					
	Estrous			Pseudopregnant		
	Rabbit no.	Esch. coli	Leukocytes	Rabbit no.	Esch. coli	Leukocytes
2	1	7.93	4.81	2	8.18	4.03
	3	8.69	5.06	4	8.23	3.88
	5	7.70	4.22	6	8.36	4.40
	7	8.40	4.10	8	8.24	4.35
	Mean	8.18	4.55	..	8.25	4.16
4	9	5.74	7.17	10	7.90	6.42
	11	5.63	8.05	12	8.43	6.68
	13	6.33	7.07	14	8.18	6.70
	15	5.76	7.05	16	8.67	5.34
	17	5.88	7.26	18	8.37	5.50
	19	5.75	7.48	20	8.69	5.46
	Mean	5.85	7.35	..	8.37	6.02

[a] The mean log number of *Esch. coli* in the inoculum per uterine horn was 8.30 (200,000,000).

Results and Discussion. The log numbers of *Esch. coli* and leukocytes recovered from the inoculated uterine horns are listed (table 1). The means of the log numbers of *Esch. coli* recovered from the 4 groups of rabbits, with the corresponding number in parentheses, are as follows: 2-hour estrous, 8.18 (151,000,000); 2-hour pseudopregnant, 8.25 (178,000,000); 4-hour estrous, 5.85 (708,000); 4-hour pseudopregnant, 8.37 (234,000,000). The difference in numbers of organisms recovered from uteri of estrous and pseudopregnant rabbits was significantly greater ($P<0.01$) at 4 hours post-inoculation than at 2 hours, the difference being only slight at 2 hours, but very pronounced at 4 hours. The relative numbers of *Esch. coli* recovered from the estrous rabbit uteri at the two time intervals suggested an initial lag in the effectiveness of the uterine defense mechanism followed by rapid destruction or elimination of the introduced bacteria. The uteri of the pseudopregnant rabbits failed totally to exhibit this effective defense mechanism.

The means of the log numbers of leukocytes (mostly polymorphonuclear) flushed from the uterine lumens of the 4 groups of rabbits were: 2-hour estrous, 4.55 (35,500); 2-hour pseudopregnant, 4.16 (14,500); 4-hour estrous, 7.35 (22,400,000); 4-hour pseudopregnant, 6.02 (1,050,000). The increase between 2 and 4 hours was significantly greater ($P<0.01$) in the estrous rabbits than in the pseudopregnant. The log number of leukocytes was subtracted from the log number of *Esch. coli* for each animal, all of the resulting figures being positive except for those in the 4-hour estrous rabbit group; statistical analysis of the figures showed a significant interaction ($P<0.01$) among the 4 groups, thus indicating an overall association between leukocytic migration into the uterine lumen and bactericidal activity of the uterus. This association, however, was true only for the estrous rabbits; the lesser influx of leukocytes into the uterine lumens of pseudopregnant rabbits between 2 and 4 hours was not associated with any apparent bactericidal activity in the uterus.

The results of this experiment indicate that leukocytic response to uterine inoculation is greater in estrous than pseudopregnant rabbit uteri during the early stages of the inflammatory response, particularly during the time interval post-inoculation that the uteri of estrous rabbits become highly bactericidal.

Microscopic examination of stained uterine sections indicated that the number of polymorphonuclear leukocytes infiltrating the endometrium was roughly proportional to the number recovered from the uterine lumen. Moderate numbers of polymorphs were present in the endometria of estrous rabbits 2 hours post-inoculation, although relatively few were recovered from the uterine lumens at that time interval. By 4 hours the endometria of estrous rabbits were infiltrated profusely. Few leukocytes could be found in the pseudopregnant rabbit endometria 2 hours after inoculation and only slight infiltration was noted at 4 hours.

The difference between estrous and pseudopregnant rabbits in the rate of leukocytic response during the early stages of an induced infection may be related to the observation that estrous rabbit uteri are normally infiltrated by more polymorphs than are pseudopregnant rabbit uteri (McDonald *et al.*, 1952). However, the differential leukocytic response may also indicate an endocrine-controlled difference in sensitivity of the uterus to an irritant or in the ability to respond to it.

At later stages of uterine inflammation, larger quantities of pus have been reported to be present in uteri of pseudopregnant than estrous rabbits; it would seem likely from the previous observations and those reported here that the rate of leukocytic influx is accelerated in inoculated uteri of pseudopregnant rabbits after the introduced bacteria have been destroyed and the rate of leukocytic influx quite possibly reduced in estrous rabbits, thus causing a reversal in the numbers of leukocytes present in the uterine lumens of the two kinds of rabbits.

Examination of the stained debris flushed from the uteri revealed a close

association between the *Esch. coli* plate counts and the number of bacteria free in the uterine lumen; only occasional bacteria were seen in the uterine debris of estrous rabbits killed 4 hours post-inoculation, while debris from the other groups contained large numbers of bacteria. However, the number of phagocytized bacteria discernible in the leukocytes flushed from the 4-hour estrous rabbit uteri could not account for the bactericidal activity of these uteri. Rather, a greater number of ingested bacteria could be seen in the leukocytes from the 4-hour pseudopregnant rabbits. The actual percentage of leukocytes which could be seen to contain bacteria was small in all groups. These observations suggested that some factor other than phagocytosis, but associated with leukocytic influx, might be at least partly responsible for the differential bactericidal activity of estrous and pseudopregnant rabbit uteri. Therefore, leukocyte-free uterine exudate from previously inoculated estrous rabbits was tested for a bactericidal effect in inoculated uteri of pseudopregnant rabbits.

Experiment 2. Effect of Estrous Rabbit Uterine Exudate in Pseudopregnant Rabbit Uteri

Methods. Each uterine horn of an estrous rabbit was inoculated with a suspension of *Esch. coli* and the rabbit killed 4 hours later. One uterine horn of each rabbit was flushed with 10 ml. of saline and an estimate obtained of the number of *Esch. coli* and leukocytes recovered, in order to ascertain the response of the exudate-donor rabbit to the inoculum. The other horn as flushed as thoroughly as possible with 1 ml. of saline and the leukocytes removed from the flushings by centrifugation. The supernatant, containing the uterine secretions and exudates, was injected along with an *Esch. coli* inoculum into one ligated uterine horn of a pseudopregnant rabbit. The exudate recipient rabbit was killed 4 hours later and the numbers of *Esch. coli* and leukocytes recovered were compared to the numbers recovered from one inoculated control horn of another pseudopregnant rabbit. Results were obtained from 8 replicates.

Results and Discussion. The log numbers of *Esch. coli* recovered from 1 horn of the exudate-donor estrous rabbits and from the exudate-infused and control horns of pseudopregnant rabbits are given in table 2. The mean of the log numbers recovered from one uterine horn of the exudate-donor estrous rabbits was 6.20 (1,580,000). All of these rabbits responded typically to the inoculum with the possible exception of the rabbit in replicate 2, from which relatively high number of bacteria were recovered.

The mean log number of *Esch. coli* recovered from the exudate-infused horns of pseudopregnant rabbits was 7.03 (10,700,000) and from the control horns, 8.60 (398,000,000). The within-replicate difference between the log numbers was significant statistically ($P<0.01$), thus indicating an effect of leukocyte-free uterine exudate from estrous rabbits on the number of bacteria recovered from inoculated uteri of pseudopregnant rabbits.

The mean log number of leukocytes recovered from the inoculated uterine horns are as follows: estrous rabbits (one horn only), 7.34 (21,900,000); exudate-infused pseudopregnant rabbits, 7.08 (12,000,000); and control pseudopregnant rabbits, 5.94 (871,000). The leukocytic response in the uteri of the estrous rabbits and the pseudopregnant controls was very similar to that of the 4-hour estrous and pseudopregnant rabbits

TABLE 2. LOG NUMBERS OF *ESCHERICHIA COLI* RECOVERED FROM INOCULATED HORNS OF UTERINE EXUDATE-DONOR ESTROUS RABBITS AND FROM INOCULATED EXUDATE-INFUSED AND CONTROL HORNS OF PSEUDOPREGNANT RABBITS [a]

	Type of rabbit					
	Estrous exudate-donor [b]		Pseudopregnant exudate-infused		Pseudopregnant control	
Replicate	Rabbit no.	Esch. coli	Rabbit no.	Esch. coli	Rabbit no.	Esch. coli
1	1	5.78	2	6.79	3	7.76
2	4	7.64	5	9.02	6	9.02
3	7	6.11	8	5.49	9	8.91
4	10	6.12	11	6.63	12	8.71
5	13	6.07	14	8.51	15	8.88
6	16	5.84	17	7.00	18	8.44
7	19	6.09	20	7.72	21	8.79
8	22	5.94	23	5.08	24	8.28
	Mean	6.20	..	7.03	..	8.60

[a] The inoculum per uterine horn varied between log numbers of 8.35 and 8.44; the mean was 8.40 (251,000,000).

[b] The exudate was obtained from the horn other than the one used for estimating the number of *Esch. coli* recovered.

in the first experiment. The number of leukocytes recovered from the exudate-infused horns suggested that the exudate from the estrous rabbits stimulated the leukocytic response and thus possibly increased the effectiveness of the recipient rabbits' own defense mechanism. Therefore, a test was made to determine whether such exudate from estrous rabbits exhibit bactericidal properties *in vitro*, and whether such properties were present in flushings from uninoculated uterine horns.

Experiment 3. In Vitro Bactericidal Activity of Uterine Exudate.

Methods. One uterine horn of 6 estrous rabbits was ligated and inoculated with *Esch. coli.* Four hours after inoculation, the rabbits were killed and each uterine horn, inoculated or uninoculated, was flushed with 1 ml. of saline. The leukocytes in the flushings from the inoculated horns were removed by centrifugation at 800 RPM for 10 minutes (International centrifuge, size 1) and the supernatant decanted into 10 x 75 mm. test tubes. This supernatant containing the uterine exudates of the inoculated

horns was inoculated with 0.2 ml. of a freshly prepared suspension of *Esch. coli;* the flushings from the uninoculated horns and saline equal in volume to the flushings (approximately 1 ml.) were also inoculated. The tubes were placed in an incubator for 2 hours at 37° C., the contents then diluted, plated and the number of live *Esch. coli* determined.

Results and Discussion. The log numbers of *Esch. coli* in the inoculum per tube and the numbers alive in each medium after the incubation period are listed in table 3. The mean of the log numbers alive after incubation in the flushings from previously inoculated uterine horns was 6.30

TABLE 3. LOG NUMBERS OF *ESCHERICHIA COLI* ALIVE AFTER INCUBATION *IN VITRO* FOR 2 HOURS IN SALINE OR LEUKOCYTE-FREE EXUDATE FROM INOCULATED[a] AND UNINOCULATED UTERINE HORNS OF 6 ESTROUS RABBITS

		In vitro incubation medium		
Replicate	Inoculum per tube	Exudate from inoculated uterine horn	Exudate from Uninoculated uterine horn	Saline
1	8.50	5.59	8.34	8.35
2	8.42	6.63	8.11	8.30
3	8.22	5.16	7.72	7.92
4	8.22	7.76	7.74	7.93
5	8.42	5.90	8.10	8.26
6	8.41	6.74	8.09	8.19
Mean	8.36	6.30	8.02	8.16

[a] The mean log number of *Esch. coli* in the inoculum per horn was 8.32 (209,000,000).

(2,000,000), after incubation in the uterine flushings of the uninoculated horns, 8.02 (105,000,000), and after incubation in saline, 8.16 (145,000,000). The within-replicate log difference between the numbers surviving in flushings from inoculated uterine horns and in saline was significant ($P<0.01$), indicating that exudate from inoculated uteri of estrous rabbits contained a substance or substances capable of direct bactericidal activity. These results tend to rule out the possibility that uterine exudate of inoculated estrous rabbits was bactericidal in pseudopregnant rabbit uteri only by stimulating the recipient rabbits' own defense mechanisms.

The difference between numbers of bacteria surviving in the flushings of inoculated and uninoculated horns was significant ($P<0.01$), and the difference in numbers surviving in flushings from uninoculated horns and in saline was not great; these two comparisons suggest that the bactericidal factor in exudate from inoculated uteri of estrous rabbits is not normally present in the uterus to any great degree.

A test was conducted next to determine whether a difference could be detected in the bactericidal properties of exudates from inoculated uteri of estrous and pseudopregnant rabbits.

Experiment 4. Comparative Bactericidal Activity of Uterine Exudates from Estrous and Pseudopregnant Rabbits

Methods. Eight replicates of one estrous and one pseudopregnant rabbit each were used. Each uterine horn was ligated at both ends and inoculated with *Esch. coli.* The rabbits were killed 4 hours later. One uterine horn of each animal was flushed with 10 ml. of saline and the flushings used to estimate the numbers of bacteria recovered from that horn, thus obtaining a measure of the bactericidal activity of that rabbit's uterus. The other horn was flushed carefully with 1 ml. of saline, the leukocytes removed by centrifugation at 800 RPM for 10 minutes (International centrifuge, size 1) and the supernatants centrifuged at 14,000 G for 30 minutes to remove the bacteria from the flushings; the difference in numbers of bacteria present in flushings from estrous and pseudopregnant rabbits would have interfered with the test for comparative bactericidal properties. Centrifugation was used to remove the bacteria from the uterine flushings since filtration tended to reduce considerably the bactericidal properties of uterine exudate from estrous rabbits. An aliquot of 0.1 ml. was removed from each supernatant after the high-speed centrifugation, diluted and plated to determine the total number of *Esch. coli* in each supernatant before inoculation with known numbers of organisms. The supernatants, containing the uterine secretions and exudates, were then inoculated with a fresh suspension of *Esch. coli* and incubated at 37° C. for 2 hours. Saline was also inoculated and incubated. The incubation media were then diluted, plated and an estimate obtained of the number of *Esch. coli* alive after the incubation period.

Results and Discussion. The log numbers of *Esch. coli* recovered from one uterine horn of the exudate-donor estrous and pseudopregnant rabbits are listed in table 4, along with the numbers alive after incubation in uterine exudate of the other horn. The mean of the log numbers recovered from one uterine horn of the exudate-donor estrous rabbits was 6.10 (1,260,000) and from the exudate-donor pseudopregnant rabbits; 8.03 (107,000,000). The means of the log numbers of *Esch. coli* alive after incubation in leukocyte-free uterine exudate of these rabbits or in saline were: estrous rabbit uterine exudate, 7.02 (10,500,000); pseudopregnant rabbit uterine exudate, 7.91 (81,300,000); saline, 8.25 (178,000,000). The within-replicate log difference between the numbers alive after incubation in uterine exudates of estrous and pseudopregnant rabbits was significant ($P<0.01$), suggesting a difference in the bactericidal properties of uterine exudates from the two kinds of rabbits 4 hours after experimental inoculation. The difference between estrous rabbit uterine exudate and saline was significant ($P<0.01$), substantiating the results of the previous experiment. The difference between numbers alive in uterine exudate of pseudopregnant rabbits and in saline was also significant ($P<0.05$), suggesting that flushings from at least some inoculated pseudopregnant rabbit uteri possess bactericidal activity 4 hours after inoculation. The leukocytic

response was much more pronounced in the estrous rabbits, typical of the comparative 4-hour response noted earlier. The bactericidal activity of the uterine exudate seems to be related closely to the degree of leukocytic influx.

The numbers of *Esch. coli* remaining in the supernatants of the centrifuged flushings did not contribute to the difference in numbers alive in

TABLE 4. LOG NUMBERS OF *ESCHERICHIA COLI* RECOVERED FROM INOCULATED HORNS OF UTERINE EXUDATE-DONOR ESTROUS AND PSEUDOPREGNANT RABBITS AND THE NUMBERS ALIVE AFTER INCUBATION *IN VITRO* FOR 2 HOURS IN SALINE OR UTERINE EXUDATE

			In vitro			
				Incubation medium		
	Type of exudate-donor rabbit[a]			Rabbit uterine exudate		
Replicate	Estrous	Pseudo-pregnant	Inoculum per tube	Estrous	Pseudo-pregnant	Saline
1	5.76	8.48	8.44	6.62	7.82	8.21
2	6.06	8.29	8.40	6.89	8.17	8.23
3	5.99	8.39	8.43	6.99	8.13	8.22
4	6.09	7.88	8.42	8.18	8.14	8.26
5	5.93	6.53	8.41	6.92	7.11	8.19
6	7.04	8.52	8.43	8.07	8.20	8.27
7	5.90	8.31	8.42	6.14	7.90	8.29
8	6.03	7.83	8.47	6.38	7.78	8.31
Mean	6.10	8.03	8.43	7.02	7.91	8.25

[a] The exudate was obtained from the uterine horn other than the one used for estimating the number of *Esch. coli* recovered. The inoculum per horn varied between log numbers of 8.34 and 8.47; the mean was 8.41 (257,000,000).

the uterine flushings of estrous and pseudopregnant rabbits after inoculation and incubation. The total numbers in the supernatants prior to inoculation were less than 1,000 in all cases except replicate 6; the supernatants of the estrous and pseudopregnant rabbit uterine flushings in this replicate contained 12,300 and 1,200 *Esch. coli*, respectively.

The results of these experiments indicate the presence of a bactericidal substance in the inoculated uteri of estrous rabbits 4 hours post-inoculation, a time when the uteri of these rabbits possess a high degree of bactericidal activity; inoculated uteri of pseudopregnant rabbits apparently contain less of the active factor at the same time interval. This bactericidal substance may be responsible in some measure for the difference in bactericidal activity of estrous and pseudopregnant rabbit uteri.

Summary

Uterine horns of estrous and pseudopregnant rabbits were ligated and inoculated with *Esch. coli*. An association was found in the estrous rabbits

between leukocytic migration into the uterine lumen and bactericidal activity of the uterus during the early stages of the induced infection. Leukocytic response was slower in pseudopregnant rabbits and was not associated with apparent bactericidal activity. Microscopic examination of cellular debris from the inoculated uteri of the estrous rabbits failed to disclose significant numbers of phagocytized bacteria; therefore, leukocyte-free exudate from previously inoculated uteri of estrous rabbits was injected into inoculated uterine horns of pseudopregnant rabbits to test for a bactericidal effect of the exudate. Fewer bacteria were recovered from the exudate-infused horns than from control horns. However, the uterine exudate from the estrous rabbits may have stimulated the uterine defense mechanism of the pseudopregnant rabbits, as suggested by increased leukocytic influx into the infused horns. The exudate was then found to possess direct bactericidal activity *in vitro*. Washings from uninoculated uterine horns of estrous rabbits were not bactericidal *in vitro*. Leukocyte-free exudates from previously inoculated uteri of estrous and pseudopregnant rabbits were compared *in vitro* in regard to bactericidal properties; the exudate from estrous rabbits was more bactericidal. These results suggest that a difference in the amount of a bactericidal substance in uterine exudates may be at least partially responsible for the differential bactericidal activity of estrous and pseudopregnant rabbit uteri.

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EFFECTS OF INBREEDING, SELECTION, LINECROSSING AND TOPCROSSING IN SWINE. I. INBREEDING AND SELECTION [1]

G. E. BRADFORD,[2] A. B. CHAPMAN AND R. H. GRUMMER [3]

University of Wisconsin, Madison

A RENEWED interest in inbreeding as a means of improving livestock followed the rapid expansion of knowledge of genetic principles and the success of hybrid corn. This was one of the factors leading to the development of cooperative research on swine breeding methods between the U.S.D.A. and several midwest state experiment stations. Most of the efforts of the workers in this Regional Swine Breeding Laboratory during the first 15 years were devoted to developing and testing inbred lines, in order to measure the effects of inbreeding, the effectiveness of selection in offsetting the effects of inbreeding, and the performance of linecross and topcross offspring of the inbred parents.

The Wisconsin Station developed and tested in various crosses five inbred lines, namely, three Chester White, one Yorkshire and one based on a cross of these two breeds. This report summarizes the data from this project on the effects of inbreeding and of the selection practiced in conjunction with this inbreeding. These data have not been included in any of the published summaries of results, with the exception of a part of the data on automatic and deliberate selection which appears in the report by Dickerson *et al.* (1954).

Materials and Methods

Animals Used. The experiment was started in the spring of 1942 when records were taken on a part of the University of Wisconsin purebred Chester White herd. Three boars, one of which had been raised in the herd, were represented by progeny. One new boar was purchased in each of the three succeeding years, and the herd was then closed to outside breeding. In 1945, the group was divided into three lines. Three boars and six sows appeared in the ancestry of these lines. The progeny of the other three boars were discarded because of defective type and inherited abnormalities. The three lines developed traced to the boars named Model A, Purdue's Moderator, and Alfalfa King, and were designated MA, PM and AK, respectively.

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[2] Present address: Department of Animal Husbandry, University of California, Davis, California.

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A group of Yorkshires was acquired in 1945, and these were inbred to form a fourth line (Y). This line was descended entirely from one male and two females. A mating between a 38% inbred son of PM and an outbred Yorkshire sow in the fall of 1946 produced the foundation of a fifth line (CY). In 1947, a small number of animals of a highly inbred (60–75%) Chester White line was acquired from the U.S.D.A. Research Center, Beltsville, Maryland. This line, denoted the B line, was used in crossing to about the same extent as the other five lines, but since few inbred litters were farrowed in this herd, no data on the line as inbreds are included.

The inbreeding was continued through 1949, during which time the herd was expanded to about 70 breeding females. In 1950, linecrosses involving the six lines in all possible combinations, as well as inbreds, were farrowed. In 1951, the litters farrowed were 2-line crosses (2LX) as in 1950, 3-line crosses (3LX), produced by mating 2LX gilts to an inbred boar of a third line, 4-line crosses (4LX), out of 2LX gilts and sired by an unrelated 2LX boar, and 2LX inbred $(2LX)^2$, produced by mating 2LX gilts to a 2LX boar of the same combination, in most cases a littermate.

All litters on which data are presented were farrowed in February, March, April and early May. In the first four years of the experiment a few litters were farrowed in the fall, but pigs from these were not used in the development of the lines except in the case of the first generation Yorkshires. Fall farrowing was discontinued in an attempt to aid in controlling disease, particularly enteritis, which was a serious problem throughout most of the experiment.

Data Recorded. The following information was recorded on all pigs in the experiment; total number farrowed per litter, number per litter at 56 and 154 days, and weight of each pig at 56 and 154 days. Litter weights at the two ages and percent survival to five months were computed. A selection index was calculated for each pig.[1] In 1948 and 1949, information

[1] $I = WI_{154} + \frac{N_{154}}{N_{154}+2}(\overline{WI}_{154} - \overline{\overline{WI}}_{154}) + 1.5(N_F - \overline{N}_F) + (N_W - \overline{N}_W) + 2\left(\frac{WL_W - \overline{WL}_W}{30}\right)$

Where: WI_{154} = weight of the individual pig at 154 days
N_{154} = number of pigs in that litter at 154 days
$\overline{WI}_{154}$ = average WI_{154} for that litter
$\overline{\overline{WI}}_{154}$ = herd average WI_{154}
N_F, N_W and WL_W = number farrowed, number weaned and total litter weight at weaning, respectively, for that litter
$\overline{N}_F$, $\overline{N}_W$ and $\overline{WL}_W$ = herd averages for these traits

Constant values of 108, 7, 5, and 122 were used after 1945 for $\overline{\overline{WI}}_{154}$, $\overline{N}_F$, $\overline{N}_W$ and $\overline{WL}_W$, respectively. No adjustment for age of dam was made in computing the indexes, but selections were made separately from the groups farrowed by gilts and by sows in each line-season.

on the age at puberty (first heat for gilts and first breeding for boars—tested on estrogenized gilts) of the pigs of each line were recorded. Results of these studies have been published (Warnick *et al.*, 1951; Wiggins *et al.*, 1951).

Inbreeding coefficients were calculated by the modification of Wright's (1922) method proposed by Cruden (1949).

Adjustment of the Data for Age of Dam Effect. Factors for adjusting sow data to a gilt basis were computed as the difference between the first and second litters of 52 Chester White females. First litters were farrowed at 1 year of age and second litters at 2 years of age. Outbred and inbred dams and litters were used but linecross data were not included. The average inbreeding of the first and second litters was essentially the same.

Correction factors were computed from these data since those used by Dickerson *et al.* (1954) obviously underestimated the difference between

TABLE 1. FACTORS FOR ADJUSTING OBSERVATIONS ON LITTERS AND PIGS FROM OLDER SOWS TO A GILT (1 YEAR OLD) BASIS

Trait	Present study	Dickerson et al. (1954)
Number per litter		
Farrowed	−3.4	−1.5
56 days	−2.0	−1.0
5 months	−1.8	...
Pig weight (lb.)		
56 days	−4 lb.	$\frac{\text{Adj. WL}_w}{\text{Adj. N}_w}$
5 months	−10 lb.	0
Litter weight (lb.)		
56 days	−70 lb.	$-(20+\frac{\text{WL}_w}{10})$
5 months	−260 lb.	..

gilt and sow litters in the present study. The two sets of adjustment factors are presented in table 1.

Statistical Analyses. The data were analyzed by analysis of variance, with tests of significance being made under the alternative assumptions that all elements—lines, years and sires—were random, and that years were fixed but the other elements were random. The same conclusions were reached under each assumption.

Methods of Estimating Inbreeding Effect. The effect of inbreeding of litter on performance was estimated by four methods as follows:

1. Within season comparison of inbreds and outbreds for the years 1944 to 1946. Only gilt litters were used. The differences for each season were weighted according to $\frac{n_1 n_2}{n_1+n_2}$ where n_1 and n_2 were the numbers of inbred and outbred litters or pigs, respectively (Snedecor, 1956).

2. (a) and (b) Intra-line of dam comparison of inbreds and 2-line crosses in 1950, by age of dam groups. The inbred and linecross litters were adjusted to zero difference in inbreeding of dam.

3. Comparison of inbred pigs out of linecross dams (2LX)[2] with linecross pigs out of linecross dams (3LX and 4LX combined) in 1951.

4. Intra-line, intra-season partial regression of performance on inbreeding of litter, holding inbreeding of dam constant. Data from 1944 to 1953 were included in the analysis.

TABLE 2. NUMBERS OF LITTERS FARROWED, INBREEDING COEFFICIENTS, AND MEANS AND INTRA-SEASON STANDARD DEVIATIONS OF PERFORMANCE TRAITS

Trait	Foundation Chester Whites (1942–45)				Five inbred lines (1946–50)			
	Gilts		Sows		Gilts		Sows	
No. litters	52		19		133		78	
Inbreeding								
Sire	0		0		.21		.18	
Dam	.01		0		.19		.10	
Litter	.06		0		.28		.26	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Number per litter								
Farrowed	9.4	2.6	11.8	2.4	7.7	2.5	11.8	2.8
56 days	6.1	2.4	6.7	3.3	3.1	2.7	6.7	2.8
5 months	5.5	2.5	6.6	3.3	2.6	2.5	6.0	2.7
Pig weight (lb.)								
56 days	26.2	6.7	27.2	8.0	20.1	6.4	26.3	6.3
5 months	123	25	132	25	94	29	111	25
Litter weight (lb.)								
56 days	160	62	183	91	63	58	176	78
5 months	676	343	876	434	246	241	662	332
Index	152	38	180	34	89	41	140	37

Two methods of estimating the effect of inbreeding of dam were used:

1. Intra-line, intra-season partial regression of performance on inbreeding of dam, holding inbreeding of litter constant.

2. Comparison of linecross pigs out of inbred dams (2LX) with linecross pigs out of linecross dams (3LX and 4LX combined) in 1951.

Results

Inbreeding. The numbers of litters farrowed, the inbreeding of parents and litters, and the means and standard deviations for eight performance traits for the foundation Chester Whites and the five inbred lines are given in table 2. The data are classified by age of dam in order to show the differential effect of inbreeding on performance of gilt and sow litters.

Figures 1 and 2 show the yearly changes in inbreeding and in five performance traits for these groups. Gilt data and sow data adjusted to a gilt basis have been combined in these graphs.

It is obvious from inspection of the table and the figures that performance for all traits decreased as inbreeding progressed. This decrease was most marked for litters out of gilts, and was proportionately greater for number of pigs raised per litter and total litter weight than for number farrowed and pig weights. The three Chester White lines were very similar in litter traits, as shown in figures 1 and 2. The CY and Y lines when first started were superior in litter traits to the inbred Chester lines, but their performance soon dropped to the level of the latter. It will be noted that the Y line

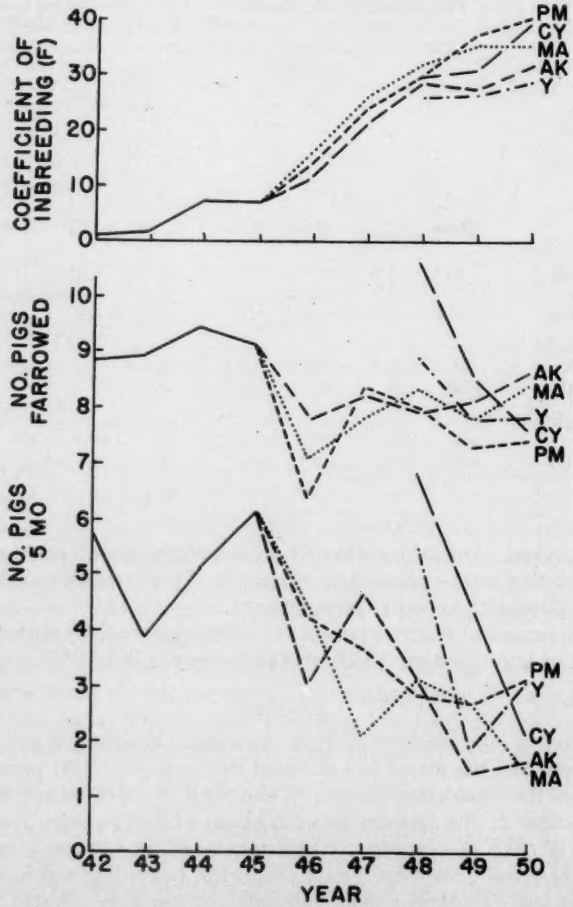

Figure 1. Time trends in inbreeding of litter and in litter size farrowed and raised to 5 months for 5 inbred lines.

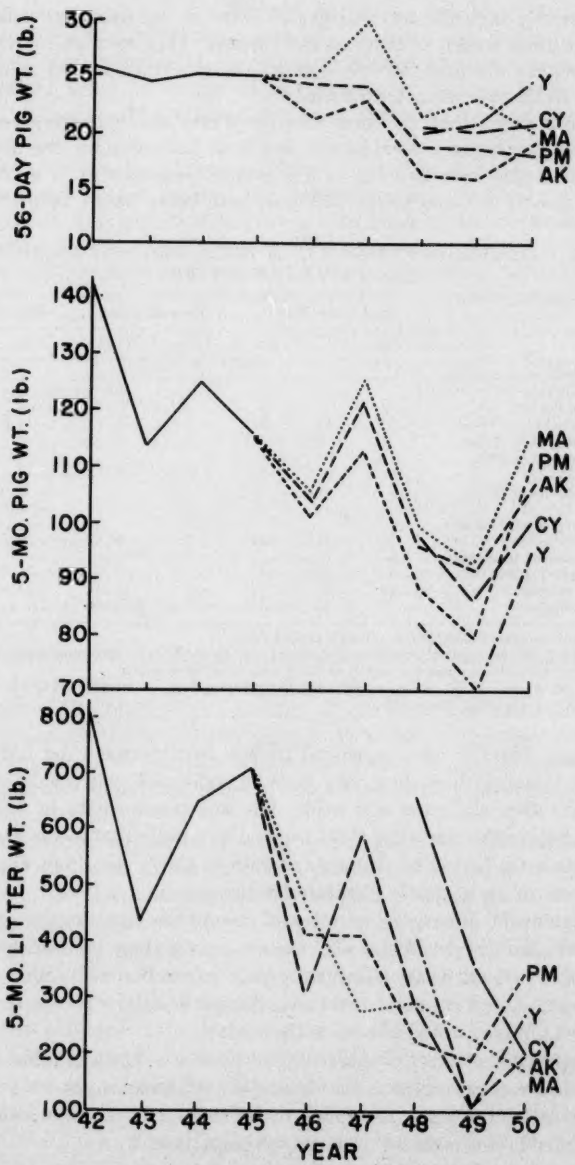

Figure 2. Time trends in individual pig weights at 56 days and at 5 months and total litter weight at 5 months for 5 inbred lines.

was relatively high for total weight of litter at weaning, but average or below for total weight of litter at five months. This was due to high mortality between weaning and five months, and to a rapid rate of gain before weaning but a slow rate after weaning.

Figure 2 shows that the three Chester White lines maintained a fairly consistent ranking in individual pig weight at both ages for the five years depicted. If gain from weaning to five months were plotted, it would show the AK line to be equal to the MA line, both being higher than the other

TABLE 3. CHANGE IN PERFORMANCE PER 10 PERCENT INCREASE IN INBREEDING OF LITTER

Item	Number per litter			Pig weight (lb.)		Litter weight (lb.)	
	Farrowed	56 da.	5 mo.	56 da.	5 mo.	56 da.	5 mo.
Method of estimation, numbers of litters and years [a]							
1. Inbreds (34) vs. outbreds (33) —1944–46	.08	—.53	—.58	—1.1	—5.1	—19.3	—91.2
2. Inbred vs. 2LX—1950							
a. Litters out of sows (15 and 23)	.34	—.27	—.54	—2.2	—9.9	—18.5	—117.8
b. Litters out of gilts (14 and 21)	—.25	—.19	—.17	1.4	—7.6	—8.5	—30.7
3. (2LX)²(16) vs. 3LX and 4LX combined (45)—1951	—.02	—.21	—.26	—2.0	—8.3	—16.5	—76.7
4. Intra-line and season regression (219)—1944–53	—.45	—.53	—.53	—.8	—4.9	—11.5	—73.2
"Average" estimates [b]	—.20	—.45	—.45	—1.1	—6.0	—14.0	—75.0
R.S.B.L. estimates [c]	—.20	—.38	—.44	.03	—3.44	.....	.....

[a] Figures in parentheses are numbers of each kind of litter.

[b] Estimate 4 and the weighted average of estimates 1, 2a, 2b and 3 given approximately equal weight. These values were used to adjust the records of the inbreds to zero inbreeding.

[c] Dickerson, *et al.* (1954). Based on intra-season difference between inbreds and 2-line crosses at four stations (not including Wisconsin).

three lines. The CY line, developed from a cross between the PM and Y lines, was higher than these two lines in individual pig weight the first two years after the cross was made, but was intermediate in 1950. The performance of this line after 1950 dropped so rapidly that it was impossible to maintain the line. The slow rate of gain of the Y line from weaning to five months of age is clearly illustrated in this graph.

No significant differences were found among the sire-progeny groups of the foundation Chester White sires nor among the three lines of this breed. Differences between years and line-by-year interaction also failed to show significance except in one or two cases. Similar results were obtained when the Y and CY lines were included in the analysis.

The estimates of effect of inbreeding of litter are shown in table 3, where each estimate is expressed as the change in performance per 10% increase in computed inbreeding. Estimates of inbreeding effect from other data are included in this table for purposes of comparison.

The estimates of the effect of inbreeding of dam are presented in table 4.

The factors used to adjust for inbreeding effects were obtained by

rounding the averages of the different estimates, each weighted approximately in accordance with the numbers on which it was based.

No tests of significance of the estimates of inbreeding effect were made in this study, since the values found are in most cases as large, and in some cases larger, than other estimates which have been shown to be significant.

Amount and Kind of Selection. Selection of boar and gilt pigs in the experiment was on the basis of the index described previously. Selection of older boars and sows was in part on the basis of the performance of

TABLE 4. CHANGE IN PERFORMANCE PER 10 PERCENT INCREASE IN INBREEDING OF DAM

Item	Number per litter			Pig weight (lb.)		Litter weight (lb.)	
	Farrowed	56 da.	5 mo.	56 da.	5 mo.	56 da.	5 mo.
Method of estimation, numbers of litters and years[a]							
1. Intra-line and season regression (219)—1944–53	.38	.12	.02	−.8	−1.5	−3.2	−1.4
2. 2LX (16) vs. 3LX and 4LX combined (45)—1951	−.80	−.51	−.53	−.2	−1.3	−16.0	−83.0
"Average" estimates[b]	0	−.10	−.10	−.7	−1.5	−6.0	−20.0
R.S.B.L. estimates[c]	−.17	−.25	−.28	.06	−.13	.....	.....

[a] Figures in parentheses are numbers of each kind of litter.

[b] Giving estimate (1) approximately three times as much weight as estimate (2) because of the larger numbers used in the former. These values were used to adjust the records of the inbreds to zero inbreeding.

[c] Dickerson *et al.* (1954). Based on Iowa data only.

their progeny and in part on the basis of their relationship to other animals in the line.

Selection differentials for a number of performance traits, including index, were calculated in order to measure the amount of selection practiced in the development of the lines. The methods used to compute these selection differentials were those given by Dickerson *et al.* (1954) with certain modifications.

The selection practiced in choosing young boars, S_1, was computed as the average deviation of the sires with progeny at one year of age, from the mean of the line, and year and age of dam group in which they were farrowed. The deviation of each sire was weighted by the number of progeny raised to five months of age. The selection practiced for older boars, S_2, was computed as the average deviation (from the group in which they were farrowed) of sires with progeny at two years of age. These values were weighted in the same manner as the S_1's. It should be noted that the S_2's are actual selection differentials rather than increments above S_1, as are the S_2's calculated by Dickerson *et al.* (1954).

The same procedure was used in calculating D_1, D_2 and D_3, the selection differentials for sows producing litters at one, two and three years of age, respectively.

These selection differentials represent the amount of selection per

generation. Since annual selection differentials are important as a measure of selection pressure per unit of time, these were calculated, as follows:

The average annual selection differential for sires ($\triangle S$) was calculated as:

$$\triangle S = \frac{N^s_1 S_1 + N^s_2 S_2}{N^s_1 A^s_1 + N^s_2 A^s_2}$$

Where: S_1 and S_2 are as defined above.

N^s_1 and N^s_2=the proportions of progeny sired by young and older boars, respectively.

A^s_1 and A^s_2=average ages of young boars (1 year) and older boars (2 years) when their progeny were born.

The same procedure was used in calculating $\triangle D$, the average annual selection differential for dams, except that the formula was extended to include N^d_3, D_3 and A^d_3 for the few cases in which sows were kept to produce litters at three years of age.

$\triangle P$, the average annual selection differential for all parents, was computed as $\frac{\triangle S + \triangle D}{2}$.

The average actual selection differentials for sires (S_1 and S_2) and dams (D_1, D_2 and D_3) and the proportion of progeny from each, for all animals produced in the experiment, are shown in table 5a. The average annual selection differentials for sires ($\triangle S$), dams ($\triangle D$) and all parents ($\triangle P$), and the average age of parents are given in table 5b.

The selection differentials for litter traits (N_F, N_W, N_{154}, WL_W and WL_{154}), given in tables 5a and 5b, have been divided by two since they are based on observations on the dam rather than on the selected individual.

The amount of selection for sires and dams based on their performance as parents in the previous year (S′ and D′, respectively) is summarized in table 5c. They were computed as deviations of the progeny of the selected older sires and dams from the mean of all pigs in the line, season and age of dam group in which these progeny were raised. The selection differentials for each parent were weighted by the number of progeny produced at the older age. It is recognized of course that the expectations of the selection differentials among sires for litter traits obtained in this manner are zero or at most small.

Some selection for litter characteristics would occur even if pigs were chosen at random from the population, due to differences in litter size. This is referred to as automatic selection. The difference between this and the total selection differential is defined as deliberate selection.

The selection differentials for boars and gilts for litter traits have been divided into automatic and deliberate portions as follows:

Automatic (A)=mean per pig at five months minus mean per litter farrowed

Deliberate (D)=mean per pig selected minus mean per pig at five months

Total (T)=A+D

TABLE 5A. SELECTION DIFFERENTIALS FOR ONE- AND TWO-YEAR-OLD SIRES (S_1 AND S_2) AND FOR ONE-, TWO- AND THREE-YEAR-OLD DAMS (D_1, D_2 AND D_3) WITH PROPORTION OF PROGENY (N) FROM EACH

Kind of parent	Prop. of progeny	Inbreeding		Number per litter			Pig weight (lb.)		Litter weight (lb.)		
		Dam	Litter	Farrowed	56 da.	5 mo.	56 da.	5 mo.	56 da.	5 mo.	Index
S_1	.51	—1.0	2.3	—.31	.44	.51	6.4	30.2	21.1	94.3	36.7
S_2	.49	3.2	4.6	—.05	.06	.06	5.1	21.1	7.4	13.9	19.1
D_1	.58	—.9	—2.7	.36	.80	.88	3.2	16.6	23.5	128.9	24.7
D_2	.34	—.8	—2.5	.27	.61	.68	2.8	16.4	17.8	127.9	24.2
D_3	.08	—2.1	—2.5	.80	.75	.93	5.2	23.8	28.9	157.6	41.6

TABLE 5B. MEAN ANNUAL SELECTION DIFFERENTIALS FOR ALL SIRES (S), ALL DAMS (D) AND ALL PARENTS (P) WITH MEAN AGE, IN YEARS, OF EACH

Kind	Age										
S	1.49	1.4	2.4	—.21	.18	.21	3.9	17.5	9.5	37.1	18.8
D	1.51	—.8	—1.8	.24	.52	.56	2.2	11.3	15.0	82.4	17.0
P	1.50	.3	.3	.02	.35	.38	3.1	14.4	12.3	59.8	17.9

TABLE 5C. SELECTION DIFFERENTIALS FOR OLDER BOARS (S') AND SOWS (D') BASED ON PROGENY PERFORMANCE

S'		—.9	—1.9	.16	.24	.21	.9	3.9	9.8	36.9	7.7
D'		.1	—.6	.12	.75	.80	.4	3.7	22.4	106.5	5.3

TABLE 5D. AUTOMATIC (A), DELIBERATE (D) AND TOTAL (T) SELECTION DIFFERENTIALS FOR BOAR AND GILT PIGS

Sex	Type of sel.										
	A	—.3	—.7	.50	1.19	1.18			31.4	138.1	
Boars	D	1.0	3.3	—1.17	—.35	—.19			10.3	51.1	
	T	.7	2.6	—.67	.85	.99			41.7	189.2	
	A	—.2	—1.0	.46	1.15	1.30			30.9	158.4	
Gilts	D	—.8	—1.8	.33	.44	.48			15.9	97.2	
	T	—1.0	—2.8	.79	1.60	1.78			46.8	255.6	

The automatic, deliberate and total selection differentials for boar and gilt pigs of the Chester White lines are summarized in table 5d. There were too few numbers in the CY and Y lines to warrant including these lines.

Effectiveness of Selection. Time trends in performance adjusted for inbreeding should give an estimate of the effectiveness of the selection applied for performance characters.

The age corrected data for four traits of all Chester White pigs in the experiment were adjusted to zero inbreeding of litter and dam by the factors given in tables 3 and 4, and the actual and adjusted figures are given in table 6. The trends in adjusted performance are shown graphically in figure 3.

TABLE 6. ANNUAL AVERAGES UNADJUSTED AND ADJUSTED TO ZERO INBREEDING FOR AGE-ADJUSTED CHESTER WHITE LITTERS

	Number per litter farrowed		Number per litter 5 months		Pig weight (lb.) 5 months		Litter weight (lb.) 5 months	
Year	Actual	Adj.	Actual	Adj.	Actual	Adj.	Actual	Adj.
42	8.8	8.9	5.7	5.7	142	142	827	834
43	9.0	9.0	3.9	4.0	114	114	440	458
44	9.5	9.7	5.1	5.5	125	130	649	723
45	9.2	9.4	6.2	6.6	116	120	715	784
46	7.0	7.3	3.9	4.6	103	112	403	516
47	8.2	8.6	3.5	4.6	119	133	428	621
48	8.0	8.6	3.0	4.5	92	111	261	520
49	7.7	8.4	2.2	4.0	87	110	172	469
50	8.1	8.8	2.1	4.0	111	133	224	546

Two points are evident from the data. First, there have been large fluctuations in environment, and secondly, selection has apparently been ineffective in offsetting the inbreeding depression effect on performance.

Discussion

Summaries of important parts of the results obtained in the Regional Swine Breeding Laboratory Project have been reported by Craft (1953) and Dickerson *et al.* (1954), and there are other publications on the results obtained at individual stations cooperating in the project. Donald (1955) has given the results of similar work in Britain. The results reported here for the Wisconsin Station will be considered in conjunction with these other results.

The level of performance of the swine in the University Experimental Swine Breeding herd was in general low throughout the experiment, even when adjusted for the presumed inbreeding effect. Disease is the most probable explanation for this, as clinical symptoms of several diseases were noted. Much of the mortality and slow growth in the 1940's was attributed to gastroenteritis in the young pigs. The use of antibiotics after 1950 appeared to result in an improvement in survival, since the performance of

the few inbreds farrowed from 1951 to 1954 was considerably higher than in previous years in spite of higher inbreeding coefficients.

One possible reason for the greater difference between gilt and sow performances in these data than at other stations is that the sows in this experiment farrowed only once a year and hence were probably in better condition at their second farrowing. Another possibility is that the combination of inbreeding and disease prevented many gilts from reaching the

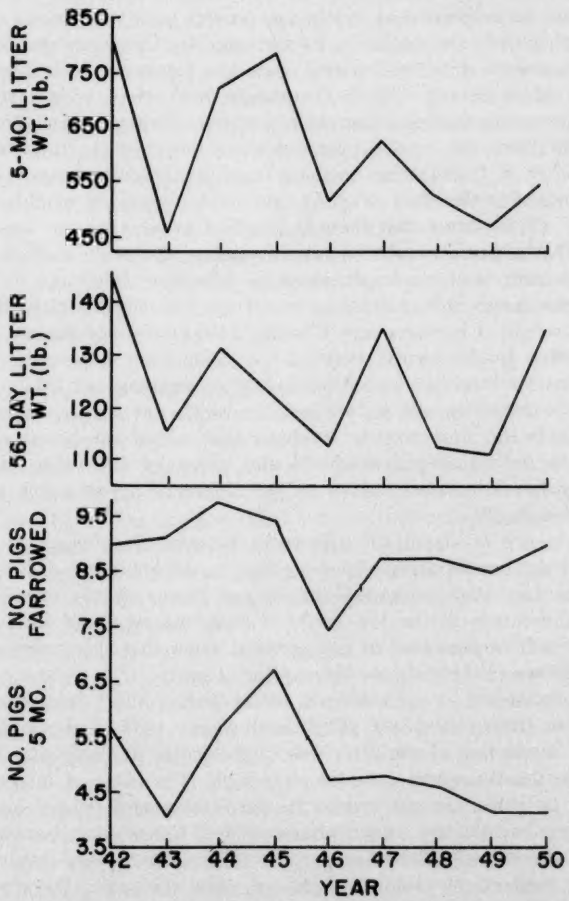

Figure 3. Time trends in performance of Chester White inbreds adjusted for inbreeding effect. Data on 3 lines combined, with all observations adjusted to a 1-year-old gilt basis.

necessary threshold in performance for normal pre- and post-natal survival and growth of their pigs. As older sows, however, they were able to produce sufficiently vigorous pigs and to provide them with enough milk that performance was more nearly normal. A high incidence of mortality of entire litters in the gilt-farrowed group compared to that in the sow-farrowed group is evidence for this hypothesis.

A third possible explanation is that the age of dam effect is not the same in inbreds and in outbreds. The factors used by Dickerson *et al.* (1954) were based on outbred data, while the present ones were based on data from both inbreds and outbreds. Factors used by Chambers and Whatley (1951) based on data from inbred sows but farrowing both inbred and linecross litters are not directly comparable to those in table 1 since the former are multiplicative rather than additive. However, the adjustment factors for litter size used by these workers are closer to those used by Dickerson *et al.* (1954) than to those found in the present study.

With regard to the effect of age of dam on individual pig weight. Dickerson *et al.* (1954) state that there is an effect at weaning but less at five months. No adjustment of five-month weights was made in their study. In the present study a larger absolute difference (although a slightly smaller percentage difference) was found for five-month weight than for weaning weight. Chambers and Whatley (1951) did not present adjustment factors for individual weights, but using their adjustment factors and means for litter size and litter weight at weaning and at six months gives adjustments for individual weights which are slightly larger than those used in the present study. Evidence that age of dam has an effect on five-month individual pig weight is also given by the value of 14 lb. found by Bernard *et al.* (1954) as the difference between sow and gilt pigs in farm herds.

The absence of significant differences between lines could be due to the small numbers of observations per line, to the low levels of inbreeding, or to the fact that environmental factors prevented the expression of genetic differences at the low levels of performance found for all lines. Evidence will be presented in a companion paper that there were actually genetic differences between the lines in rate of gain.

The comparison of the estimates of inbreeding effect from these data with those from other data (Dickerson *et al.*, 1954) indicate that the effect of inbreeding of the litter was quite similar for litter size but was greater in the Wisconsin data for pig weight. The effect of inbreeding of the dam on litter size was smaller in the Wisconsin data, but on gain it was larger than for the other stations studied. Considering, however, that different breeds and different environmental conditions were involved, and that the methods of estimation are not quite the same, the results are not widely discrepant.

The results indicate that considerable selection for performance traits was applied in the development of the lines. Major emphasis in the selec-

tion of young boars was apparently given to individual weight, which is in agreement with the finding of Dickerson *et al.* (1954). The fact that the original selection differentials for boars selected for use a second season were less than those for all boars selected for breeding, for all traits, shows that selection for these older boars was not based on the same criteria as for younger boars. Usually, the more highly inbred males were kept for a second year, and this is reflected in the higher positive S_2's for inbreeding.

Little selection was practiced for older sires on the basis of performance of their progeny, whereas sows kept to produce a second litter were in most cases substantially above the average in performance of their first litters.

Automatic selection accounted for most of the selection practiced for litter traits. Considering the average for both boars and gilts, deliberate selection was negative for number farrowed, approximately zero for number weaned, about one-tenth of the total selection for number at five months and about one-third for weight of the litter at weaning and at five months. Automatic selection was negative for inbreeding coefficients of dam and of litter, indicating the depressing effect of inbreeding on survival. Deliberate selection for levels of inbreeding was positive for boar pigs but negative for gilts. It may be noted that the automatic selection differentials, which were computed separately for boars and gilts since both sexes were not always selected from the same year and age of dam groups, were essentially the same for both sexes.

Dickerson *et al.* (1954) have presented data indicating that selection practiced in the development of inbred lines is ineffective in improving performance even when allowance is made for the computed effect of inbreeding on performance. These authors consider that this result in conjunction with other evidence points to heterozygote advantage or overdominance as the mode of action of the genes governing the traits studied. Boulware (1954) has also presented evidence indicating the ineffectiveness of selection for "heritable traits" in swine.

The results of the present experiment are in agreement with those referred to above with regard to the lack of effectiveness of selection in the development of the inbred lines.

Whether or not inbreeding in swine can be practiced without the losses encountered in this and other comparable experiments is not answerable on the basis of the results thus far. A relatively small number of inbred lines has been developed and most of them have been developed by rather mild inbreeding. It may be that within the swine population there are individuals or groups of individuals whose germplasm is such as to allow of rather intense inbreeding with decrease in heterozygosity accompanied by little or no reduction in elements of vigor. By intense inbreeding of many small groups of individuals and severe culling between groups it may be possible to isolate such superior stock.

Likewise for selection, it is not known from the studies made thus far whether the ineffectiveness is actually due to the masking of the genetic

variation by the environmental, or because of the large amount of non-additive inheritance such as overdominance or negative genetic correlation between traits.

Partial answers to these questions should be forthcoming from the swine breeding research now underway, particularly that having to do with intense inbreeding in many small groups and with recurrent reciprocal selection.

Summary and Conclusions

Data are presented on the effects of inbreeding, the amount of selection practiced, and the effectiveness of this selection, in the development of five inbred lines of swine at the Wisconsin Agricultural Experiment Station.

An increase of 10% in litter inbreeding coefficient resulted in decreases of approximately .20 pigs farrowed per litter, .45 pigs raised per litter, 6 lb. in individual pig weight at five months and 75 lb. in total litter weight at this age. The corresponding decreases for 10% increase in inbreeding of dam were 0 and .10 pigs and 1.5 and 20 lb., respectively.

The selection practiced for performance traits within the lines during their development was apparently ineffective, even after adjustment for the estimated inbreeding effect.

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EFFECTS OF INBREEDING, SELECTION, LINECROSSING AND TOPCROSSING IN SWINE. II. LINECROSSING AND TOPCROSSING[1]

G. E. BRADFORD,[2] A. B. CHAPMAN AND R. H. GRUMMER[3]

University of Wisconsin, Madison

INBRED lines of the Chester White and Yorkshire breeds were developed at the Wisconsin Experiment Station as a part of the Regional Swine Breeding Laboratory cooperative research project. These lines were tested in several combinations with each other and with outbred animals at the University farm, and in addition inbred and linecross boars from these lines and from lines developed at other experiment stations were compared with outbred boars in matings with outbred sows in two-sire farm herds.

The results of the experiment with respect to the inbreeding effect and the amount, kind and effectiveness of selection in the development of the lines are published in a companion paper (Bradford *et al.*, 1958). The present paper includes the results of the linecrossing and topcrossing tests carried out to evaluate the usefulness of these mating systems.

Materials and Methods

University Experimental Breeding Herd. The stocks and experimental procedure used in the development of the lines have been described in the paper cited above.

Two-line crosses were produced in 1950. These involved all possible combinations of the following lines: Three Chester White (MA, PM, AK), one Yorkshire (Y), and one Chester-Yorkshire (CY), developed at Wisconsin, and one Chester White (B), developed at the U.S.D.A. Agricultural Research Center, Beltsville, Md. In 1951, four groups were produced: two-, three- and four-line crosses (2LX, 3LX and 4LX) and inbred two-line crosses $(2LX)^2$, obtained by mating 2LX gilts to a 2LX boar combining the same two lines, in most cases to a litter mate. This last group was produced in order to have a means of estimating inbreeding effect, and

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[2] Present address: Department of Animal Husbandry, University of California, Davis, California.

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also as a potential foundation for new lines in case any of the combinations showed unusual promise.

In 1951, weanling purebred boars and gilts of the Duroc, Poland China and Chester White breeds were purchased. These and the inbred and linecross animals already available were mated to produce the following combinations in 1952:

Sire	Dam	Designation of litter
Purebred	Purebred-same breed	Purebred —PB
	Purebred-different breed	Crossbred —XB
	2LX	Outbred-2LX—O-LX
Inbred	Purebred-same breed	Topcross —TX_F
	Purebred-different breed	Topcrossbred —TXB_F
	2LX	Three-line cross—3LX
2LX	Purebred-same breed	Topcross —TX_{LX}
	Purebred-different breed	Topcrossbred —TXB_{LX}
	2LX	Four-line cross—4LX

In order to obtain information on the productivity of these nine groups, gilts were chosen at random from within each group to farrow in 1953. They were mated to the following kinds of boars: Outbred Duroc and Poland-China, inbred Chester White (AK and PM lines), inbred Landrace (L) and inbred Landrace Poland China (LPC). Eight individuals of each of the last two lines had been obtained from Beltsville in the fall of 1951.

Data are presented for each system of mating on the number of pigs farrowed and raised per litter and on the weights of pigs and litters at 56 and 154 days of age. Data recorded on the age of puberty of gilts from each group produced in 1950, 1951 and 1952 have been reported by Foote *et al.* (1956).

Farm Topcross Tests. The farm topcross tests were conducted over an eight-year period, the first boars being tested in the fall of 1946. These boars were placed in herds of 10 or more sows where the owner had agreed to divide his sows as evenly as possible between the experimental boar and an outbred, purebred boar of his own choosing. Identification of sows and pigs, the keeping of litter records and obtaining weights on the progeny at ages of approximately eight weeks and five months were under the supervision of assistants on the project from the University.

Durham *et al.* (1952) reported the design of this experiment and the results obtained with inbred boars from 1947 to 1950. The comparisons were continued through 1954, most of the boars tested from 1951 to 1954 being two-, three- and four-line cross boars from the Wisconsin station. The total data for the eight years are included in this report in order to compare the results obtained with linecross boars with those obtained with inbred boars, to include information on an additional trait, five-month litter weight, and to make an additional classification of the data.

The progeny of the inbred or linecross boars have been designated as topcrosses (TX) and the progeny of the outbred boars have been designated as non-topcrosses (NTX). In herds in the program for the first year, all the dams of the topcross and non-topcross litters were outbred (NTX), and thus there were, with regard to the classifications in this experiment, only two kinds of litters in these herds. In herds comparing boars in two or more consecutive years, approximately half the dams were topcross and half were non-topcross, and since these two groups were each divided between the two boars, there were four groups of litters in each of these herds. Comparisons of the progeny of the two boars have been made within type-of-dam in all herds. This type-of-dam classification was not made in the earlier study; however, the majority of the herds providing boar comparison data at that time were first-year herds.

All litters for which data are presented were farrowed by one-year-old gilts. The herds in which both the topcross and non-topcross litters were straightbred and those in which both were crossbred have been combined. These two groups comprise the main part of the data, with the latter group more numerous. In 14 of the 93 herds in the experiment, the topcrosses were crossbred whereas the non-topcrosses were non-crossbred. These 14 herds have been considered separately. There were no herds in which the non-topcrosses had the advantage of crossbreeding. The main effect of topcrossing is therefore unconfounded with the effect of crossbreeding in the data as analyzed.

As in the earlier study, data on the productivity of topcross and non-topcross gilts were obtained wherever possible. The gilt productivity comparisons have been made within kind of mate (i.e., within outbred and within inbred and linecross boars) in the same manner that comparisons of the viability and rate of gain of the topcross and non-topcross pigs were made within kind of dam.

The disproportionate numbers in these data at every level of classification made it necessary to use a method of analysis which did not assume a balanced design. The method used utilized intra-herd topcross mean minus non-topcross mean differences as the experimental observations, and weighted these in accordance with the reciprocal of their variance. The detailed method is available elsewhere (Bradford, 1955). It should be noted that the results were essentially the same whether the differences were unweighted, weighted according to the numbers of observations, or by the weighting method actually used, which gives a weight intermediate between these two.

Results

University Experimental Breeding Herd. The numbers of litters farrowed, the inbreeding of the parents and litters, and the means and standard deviations for the performance traits of the inbred and linecross groups farrowed in 1950 and 1951 are given in table 1.

TABLE 1. MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (S.D.) FOR PERFORMANCE TRAITS OF INBRED, 2-, 3- AND 4-LINE CROSS AND INBRED 2-LINE CROSS PIGS

Year	1950								1951							
Mating system	Inbred				2LX				2LX		3LX		4LX		(2LX)[a]	
Age of dam	Gilts		Sows		Gilts		Sows		Gilts		Gilts		Gilts		Gilts	
No. of litters	14		13		21		23		16		21		24		16	
Inbreeding																
Sires	.29		.31		.33		.30		.42		.37		.02		.02	
Dams	.28		.24		.29		.34		.30		.05		.05		.02	
Litters	.36		.33		.05		.04		.04		.04		.04		.32	
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.
Number per litter																
a. Farrowed	7.7	1.7	11.6	1.8	8.5	2.1	10.5	3.2	6.9	2.9	8.6	2.4	9.2	2.4	8.9	2.6
b. 56-days	2.2	2.8	6.6	2.1	2.7	2.9	6.6	3.3	4.7	2.9	6.3	3.3	5.7	3.6	5.4	2.5
c. 5 months	1.5	2.0	5.0	2.8	2.0	2.6	6.4	3.2	4.4	2.9	6.1	3.2	5.4	3.6	5.1	2.2
Weight per pig (lb.)																
a. 56 days	19.7	6.5	25.3	7.7	23.1	8.3	31.5	8.3	29.3	5.7	31.0	7.5	28.7	7.2	24.7	6.0
b. 5 months	105	23	114	22	125	34	145	28	143	20	150	27	142	26	124	25
Weight of litter (lb.)																
a. 56 days	44	56	167	81	62	75	209	110	138	90	195	102	162	104	133	67
b. 5 months	157	209	569	358	250	338	920	451	635	447	921	488	772	526	628	295
Index	104	36	140	40	147	54	203	43	182	43	207	40	193	42	147	41

TABLE 2. MEANS AND AVERAGE INTRA-MATING SYSTEM STANDARD DEVIATIONS (S.D.) FOR PERFORMANCE TRAITS OF LINECROSS, TOPCROSS, PUREBRED AND CROSSBRED LITTERS (1952)

Mating system of litter	3LX	4LX	O-LX	TX_F	TX_{LX}	TXB_F	TXB_{LX}	PB	XB	All groups	
No. of litters	8	7	9	3	4	8	11	10	9	69	
Number per litter										Mean	S.D.
a. Farrowed	9.8	8.4	7.6	6.0	9.0	7.0	7.2	6.8	7.9	7.7	2.5
b. 56 days	7.0	6.4	5.9	5.7	7.5	6.1	5.9	5.0	6.1	6.1	2.8
c. 5 months	6.1	5.9	5.3	5.3	7.0	5.9	5.4	4.8	6.1	5.7	2.6
Weight of pig (lb.)											
a. 56 days	24.6	24.8	26.4	22.4	21.7	27.2	26.4	22.9	29.8	25.6	6.9
b. 5 months	110	120	127	119	110	139	133	120	151	127	28
Weight of litter (lb.)											
a. 56 days	172	160	155	127	162	166	156	115	182	156	78
b. 5 months	674	704	676	634	770	815	715	576	921	722	366
Index	134	145	158	135	128	176	168	135	202	158	44

Results of Duncan's multiple range test applied to data on 5-month pig weight[a]

3LX	TX_{LX}	TX_F	PB	4LX	O-LX	TXB_{LX}	TXB_F	XB
110	110	119	120	120	127	133	139	151

[a] Members of an underlined subset do not differ significantly ($P>.05$) from each other.

The 1950 data show a large advantage for two-line crosses over inbreds in survival and rate of growth. These data also show a large difference between the performance of gilt and sow litters in both mating systems, since in a high proportion of gilt litters no pigs survived.

Differences among the four linecross groups in 1951 were significant only for individual pig weight at weaning and at five months. It is obvious from inspection of the means that these significant differences are due to the low performance of the $(2LX)^2$ group. The two-, three- and four-line

TABLE 4. MEANS OF PERFORMANCE TRAITS OF LINECROSS, TOPCROSS, PUREBRED AND CROSSBRED LITTERS CLASSIFIED BY BREEDING OF SIRE (1953)

Breeding of sire	Inbred				Outbred	
	PM	AK	L	LPC	Duroc	Poland
No. of litters	5	3	13	13	11	8
Number per litter						
a. Farrowed	9.2	6.0	9.4	8.8	9.1	10.9
b. 56 days	7.8	4.3	7.3	7.8	8.4	8.8
c. 5 months	7.2	4.0	7.0	7.5	8.0	8.6
Weight of pigs (lb.)						
a. 56 days	21.8	31.8	27.1	26.5	23.8	21.7
b. 5 months	104	142	130	132	120	118
Weight of litters (lb.)						
a. 56 days	170	138	198	208	199	190
b. 5 months	747	567	908	985	960	1014
Index	120	186	171	174	156	150

crosses are nearly equal in these traits. The number of pigs raised per litter by gilts farrowing two-line cross litters, while still low relative to accepted standards, was more than double that of the 1950-farrowed litters in this group, indicating a difference between these two years in environmental factors affecting performance.

Differences among the nine mating systems in 1952 (table 2) were significant only for five-month individual pig weight. Duncan's multiple range test was applied to the data for this trait to determine to which means this significance was due. The results of this test, shown at the bottom of the table, indicate that the crossbreds from outbred sires and dams (XB) were significantly superior to the five lowest groups (3LX, TX_{LX}, TX_F, PB and 4LX), that the crossbred offspring of inbred sires mated to outbred dams (TXB_F) were significantly superior to the lowest two of these (3LX and TX_{LX}) and that the other groups were not significantly different from each other, although the difference between the TXB_{LX} group and the lowest two approached the .05 probability level. The three highest groups and eight of the nine litters in the next highest

TABLE 3. MEANS AND AVERAGE INTRA-MATING SYSTEM STANDARD DEVIATIONS (S.D.) FOR PERFORMANCE TRAITS OF LINECROSS, TOPCROSS, PUREBRED AND CROSSBRED DAMS (1953)

Mating system of dam	3LX	4LX	O-LX	TX_F	TX_{LX}	TXB_F	TXB_{LX}	PB	XB	All groups	
No. of litters	4	5	7	4	4	6	7	6	10	53	
Number per litter										Mean	S.D.
a. Farrowed	9.2	7.4	8.9	9.0	10.5	8.3	11.3	8.8	9.1	9.2	2.4
b. 56 days	7.8	7.0	7.7	7.0	8.5	7.0	9.1	7.5	7.8	7.8	2.4
c. 5 months	7.8	6.8	7.4	6.2	8.2	6.8	8.9	7.2	7.2	7.4	2.5
Weight of pig (lb.)											
a. 56 days	27.6	24.0	24.0	25.2	20.0	26.5	22.8	24.2	28.3	24.9	7.1
b. 5 months	138	120	120	128	117	125	117	118	133	124	26
Weight of litter (lb.)											
a. 56 days	214	168	185	176	170	186	209	181	221	193	74
b. 5 months	1072	819	890	801	968	856	1035	842	961	919	352
Index	189	152	153	160	147	160	158	144	178	161	42

group were crossbred and the five low groups were all straightbred except for a limited number of Yorkshire-Chester crosses in the 3LX and 4LX groups.

The 1953 data, shown in table 3 classified by mating system of dam, were intended to provide information primarily on the productivity of gilts of the nine groups produced the previous year. Classification of these same data by breeding of sire is shown in table 4. This allows comparison of the different kinds of boars to which these gilts were mated.

To facilitate analysis of these data sufficient observations were discarded to balance the design. This involved the complete elimination of the AK and PM groups and randomly discarding a certain number of litters in other subclasses. An additional classification, type of sire, was then made, treating the L and LPC sires as one group and the Duroc and Poland sires as another.

An analysis of the variance into portions due to mating system of dam, type of sire, breed within type of sire, and appropriate interactions was then carried out for the following traits: Number per litter farrowed and weaned, total litter weight at weaning and at 5 months, and litter average 5-month individual pig weight.

No significant differences for any of the main effects were found, although the type-of-sire mean square for average 5-month pig weight was relatively large. The degrees of freedom for type of sire and breed within type-of-sire were 1 and 2, respectively, so that a very large difference would be required for significance. The progeny of the L and LPC boars averaged 131 lb. for this trait, compared to 119 for the progeny of the Duroc and Poland sires. The only interaction which was found to be significant at the .05 probability level was that between mating system of dam and type of sire, also for 5-month pig weight.

Farm Topcross Tests. The groups from which the boars came for the farm topcross tests and the numbers of topcross and non-topcross litters involved in each comparison are given in table 5. These numbers do not include those herds in which the topcrosses had the advantage of cross-breeding. The latter were analyzed separately.

The inbred lines represented have been divided into three groups in this table: Wisconsin lines, representing the Chester White and Yorkshire breeds; lines carrying 50% or more of Landrace breeding (nearly all of these boars were from lines carrying about 75% Landrace inheritance); and "other" lines. The linecross boars have also been divided into three groups: Wisconsin 2LX's; Wisconsin 3LX's, 4LX's and $(2LX)'s^2$; and "other" linecrosses.

The Wisconsin groups were separated in order to provide a comparison of the results of using inbred and linecross boars from the same stock. The "Landrace" lines were grouped separately because of the presumed genetic diversity of these lines compared to the others tested. The "other" groups, representing primarily Chester White and Poland China breeding in the

TABLE 5. SOURCE OF DATA FOR COMPARISON OF INBRED AND LINECROSS BOARS WITH OUTBRED BOARS

Test boar group	No. of lines or LX groups	No. of herds	No. of boars	No. of litters with data from birth to 5 months of age				No. of gilts with productivity data at one year of age			
				NTX dams		TX dams		Outbred mates		Inbred or LX mates	
				TX litters	NTX litters	TX litters	NTX litters	TX gilts	NTX gilts	TX gilts	NTX gilts
Inbreds											
Wis. lines[a]	5	22	21	107	125	46	36	54	60	47	37
"Landrace"[b]	4	15	10	77	85	27	31	41	51	22	20
Other[c]	3	10	7	49	111	12	12	35	99	27	24
Total	12	47	38	233	321	85	79	130	210	96	81
Linecrosses											
Wis. 2LX	1	12	12	45	66	37	25	28	27	31	31
Wis. 3LX, 4LX, (2LX)[2]	3	10	9	52	58	16	17	29	30	..	..
Other LX[d]	3	10	7	64	50	6	11	10	6	..	..
Total	7	32	28	161	174	59	53	67	63	31	31
Grand total	19	79	66	394	495	144	132	197	273	127	112

[a] Three Chester White, one Yorkshire and one Chester-Yorkshire.
[b] LPC (74% Landrace, 26% Poland China), seven boars; LD (76% Landrace, 24% Duroc), Minn. No. 1 (48% Landrace, 52% Tamworth), and purebred Landrace, one boar each.
[c] CWL (86% Chester White, 14% Landrace), three boars; Chester White (Beltsville), one boar; and Iowa Poland China, three boars.
[d] Nebraska Duroc, three boars; Iowa Poland China, three boars; and LPC-LLB, one boar.

case of the inbreds, and mainly Duroc and Poland China in the case of linecrosses, were considered as groups because there were insufficient numbers for any one line to characterize that line, but the overall data add to the information on topcrossing as a mating system. The Wisconsin

TABLE 6. WEIGHTED INTRA-HERD AVERAGE DIFFERENCES BETWEEN TOPCROSSES AND NON-TOPCROSSES

Test boar group	No. of herds	No. farrowed per litter	No. per litter at 5 mo.	Percent survival	5-month litter wt., lb.	5-month pig wt., lb.
A. Both groups out of non-topcross dams						
Wis. inbreds	19	—.4	—.1	2	—17	1
"Landrace" inbreds	13	.1	.2	1	91	11*
Other inbreds	10	.2	0	0	—14	—2
All inbreds[a]	42	—.1	0	1	17	3
Wis. 2LX	10	.1	.9	10*	136	7
Wis. 3LX, 4LX, $(2LX)^2$	9	.5	.4	—1	43	—1
Other LX	10	.3	—.1	—3	—4	—1
All LX[a]	29	.3	.4	2	59	2
B. Both groups out of topcross dams						
Wis. inbreds	7	—.2	—.2	2	—90	—7
"Landrace" inbreds	6	.9	.2	—5	12	—2
Other inbreds	4	—.1	—1.2	—10	—169	—2
All inbreds[a]	17	.2	—.2	—3	—63	—4
Wis. 2LX	9	—.5	.6	8	38	—4
Wis. 3LX, $(2LX)^2$	2	—.8	.8	18*	124	8
Other LX	2	—2.1	—.8	7	—68	9
All LX[a]	13	—.8	.4	10*	41	0

[a] Weighting each herd in proportion to the amount of information contributed.
* Approximate 95% confidence interval does not include zero.

2LX group was separated from and compared with the 3LX, 4LX and $(2LX)^2$ group. The last three groups were not separated because of the small numbers of boars representing each of them.

The weighted average intraherd topcross minus non-topcross differences for the groups tested are given in tables 6 and 7. Table 6 compares the performance, from birth to five months, of the progeny of the experimental and control boars, with part A representing comparisons within NTX dams and part B comparisons within TX dams. Table 7 is based on the performance of gilts of these groups when kept to produce litters at one year of age, with part A being the results of comparisons where both TX and NTX gilts were mated to outbred boars, and part B those where both groups were mated to inbred or line-cross boars. For each of the differences an approximate 95% confidence interval was computed, and any differences, positive or negative, differing from zero by more than this amount are marked with an asterisk.

The average performance for all animals in this experiment for the five traits considered was as follows: For number farrowed, 8.9, for number at 5 months, 6.8, for percent of pigs born surviving to 5 months, 76, for total litter weight at 5 months, 881 lb., and for individual pig weight at

TABLE 7. WEIGHTED INTRA-HERD AVERAGE DIFFERENCES BETWEEN TOPCROSS AND NON-TOPCROSS GILTS

Sire of TX gilts	No. of herds	No. farrowed per litter	No. per litter at 5 mo.	Percent survival	5-month litter wt., lb.	5-month pig wt., lb.
A. Both groups mated to outbred boars						
Wis. inbreds	10	.7	1.2	7	170*	4
"Landrace" inbreds	8	1.4*	.9	−2	172	6
Other inbreds	9	.4	.8	6	128	5
All inbreds[a]	27	.8*	1.0*	4	156*	5*
Wis. 2LX	5	.2	−.4	−3	−16	4
Wis. 3LX, 4LX	3	.2	−.3	−4	−64	−3
Other LX	2	1.3	.7	−4	71	−3
All linecross[a]	10	.4	−.2	−3	−18	0
B. Both groups mated to inbred or linecross boars						
Wis. inbreds	10	.1	−.1	−3	−6	2
"Landrace" inbreds	5	1.0	1.0	3	119	−1
Other inbreds	7	.1	−.5	−4	−58	0
All inbreds[a]	22	.3	0	−2	5	0
Wis 2LX	5	−1.0	−1.4	−6	−104	12*

[a] Weighting each herd in proportion to the amount of information contributed.
* Approximate 95% confidence interval does not include zero.

this age, 130 lb. The differences in these tables may therefore be related to these means to estimate the relative change in performance.

Three groups of topcrosses were significantly superior to their controls in one or more traits. These were the topcrosses by "Landrace" boars, for 5-month pig weight; those by Wisconsin 2LX boars, for viability; and the topcross gilts by all inbred boars, in numbers of pigs farrowed and raised and weight of pigs and litters at 5 months. The progeny of linecross boars out of topcross dams had a significantly higher viability than their controls, but since this was accompanied by a smaller initial litter size it would appear to be of less practical significance than the others. It may be noted that in general the comparisons where both groups were out of topcross dams show much less favorable results for the topcross pigs than those in which both groups were out of NTX dams.

The results obtained in herds in which the topcrosses were crossbred and the non-topcrosses were purebred showed a much greater advantage for topcrosses than did those shown in the above tables.

The effect of using inbred and linecross boars on variability of the off-

spring is shown in table 8. These mean squares were estimated from the progeny of non-topcross dams only.

There appears to have been little effect on variability in litter traits from the use of inbred or linecross boars. The analysis of 5-month individual pig weight was not done separately for herds in which inbred and linecross boars were tested. The mean square for this trait for both groups of topcrosses was 493 (2971 d.f.), and for the non-topcrosses in the same herds it was 544 (4009 d.f.). A difference of this size on these degrees of freedom is on the border line of significance.

TABLE 8. WITHIN HERD MEAN SQUARES FOR TOPCROSS AND NON-TOPCROSS

Source of estimate	Degrees of freedom	N_F	N_{154}	Percent survival	WL_{154}
Topcross litters by inbred boars	218	4.6	4.2	414	74654
Non-topcross litters in same herds	302	3.8	4.0	329	66473
Topcross litters by linecross boars	151	4.1	3.3	238	60016
Non-topcross litters in same herds	161	4.2	3.6	295	62753

Discussion

It appears from this and other experiments that linecrossing results in a recovery of the vigor lost through inbreeding. Whether the performance of linecrosses is superior to that of outbreds is less clear. Robison, (cited by Craft, 1953), Warwick and Wiley (1950) and Chambers and Whatley (1951) have reported results indicating that two-line crosses are about equal to outbreds in total litter weight at five or six months, being somewhat below outbreds in litter size due to inbreeding of the dams, but above the outbreds in individual pig weight. Three-line crosses or rotational crosses were found, in each of the experiments cited, to be superior to outbreds in both litter size and gain and therefore much superior in total litter weight. Dickerson *et al.* (1946) concluded from results at the Iowa station that at least the better combining lines could be used to produce linecrosses superior to outbreds in performance. Results from Minnesota (Sierk and Winters, 1951) led these authors to the same conclusion. Contemporary outbred controls were not available in these last two cases.

The results of linecrossing in the present experiment show little advantage over outbreeding for this mating system. Outbred controls were not available in 1950 and 1951, but the performance of the two-, three- and four-line crosses in those years does not suggest that they would have been much if at all superior to outbreds. In 1952, when outbred controls were produced, two-line crosses were not produced, but the performance of the three- and four-line crosses was about the same as that of the outbreds. The results of the 1952 trial show clearly the advantage of crossbreeding,

but indicate that this may be realized as fully by crossing non-inbreds of different breeds as by crossing inbreds of different breeds. This should be kept in mind when evaluating the results of such studies as those reported by Craft (1953) and Sherritt *et al.* (1954). The results of the farm topcross tests also emphasize the necessity for separating topcrossing and crossbreeding effects.

Since the topcross differences showing significance at the .05 probability level represent only a small proportion of the total comparisons, it may be that these represent only normal variation from a mean difference of zero. However, the consistency of the results within at least two of the three groups referred to above suggest that the differences are real.

In the "Landrace" group, four lines were represented. The average advantage in favor of the topcrosses for 5-month pig weight for this group was 11 lb., with the differences for the four lines separately being 10.6, 11.9, 11.1 and 12.7 lb. In addition to this, results from the University farm in 1953 showed a difference of 12 lb. in favor of the progeny of L and LPC line boars compared to progeny of outbred Duroc and Poland sires. Hazel *et al.* (1948) also reported a 12-lb. advantage for topcrosses by inbred Landrace boars. It is not possible to state whether these favorable results are due to inbreeding and selection, or to the superiority of the foundation Landrace stock and/or the genetic diversity of this breed compared to American breeds. The latter possibilities seem more likely, since few other lines have shown this advantage.

The results of the comparisons of topcross gilts by inbred boars also are consistent within the group. The differences in number of pigs per litter at 5 months and total 5-month litter weight were in favor of the topcross gilts for each of the 11 lines represented in the comparisons in which both topcross and non-topcross gilts were mated to outbred boars. The actual differences for litter size ranged from +.04 to +2.52, averaging 1.0 pig, with 10 of the 11 lines showing an advantage exceeding 0.5 pig. For 5-month litter weight, the values ranged from +22 to +355 lb, averaging 156 lb. This appears then to be a general effect of the mating system, since the 11 lines represented at least four different breeds.

The third group showing a favorable trend were the topcrosses from the Wisconsin two-line cross boars. Progeny of 10 boars of this group showed a significant average increase of 10% in viability over their non-topcross controls and increases of 7 and 136 lb. in 5-month pig and litter weights, respectively. These approached significance. This differs from the results for these traits obtained with boars of the lines from which these linecrosses came, from those obtained with other linecrosses from these lines and from those with linecrosses from other breeds. Progeny of each of the last three groups were almost identical in performance to the non-topcrosses for all traits. If the difference between the different groups is real, the reason for it is not clear. Epistasis is a possible explanation. There were no direct comparisons between these different kinds of boars in the

farm herds, but since there were similar controls for each group, the results should be comparable. Further experimental work on this point to determine if these results are reproducible would be desirable. A comparison of inbred and linecross boars in topcrossing was conducted one year in the University herd and the results do not show the same trend as the farm topcross tests. However, there were small numbers of boars and litters and the farm results should give a more reliable picture.

The results of the gilt productivity comparisons for the inbred and linecross boars also differ. While the topcross gilts by inbred boars showed a consistent advantage, which was significant over all lines, no advantage was shown by topcross gilts from linecross boars. Gilts by Wisconsin inbred boars (10 herds) raised litters 170 lb. heavier at 5 months than did non-topcross gilts in the same herds. The comparable figure for Wisconsin two-line cross boars (5 herds) was —16 lb., and for Wisconsin three- and four-line crosses (3 herds) was —64 lb.

The reason the comparisons within topcross dams show the topcross pigs with less advantage (or greater inferiority) than in comparisons within non-topcross dams is not clear. The same trend shows in the productivity comparisons; those in which both kinds of gilts were mated to inbred or linecross boars showed less advantage for the topcross gilts than those in which both were mated to outbred boars. It is unlikely that the progeny of topcross gilts mated to outbred boars had reached a level of performance upon which improvement could not be made. There were insufficient numbers of specific combinations of lines to determine if line differences were a factor. The phenomenon appears to be general, showing in the comparisons in which the topcrosses had the advantage of crossbreeding.

Inbreeding suggests itself as a possible explanation, but inbreeding was avoided by testing only unrelated boars in the same herd in consecutive years. It may be, however, that the selection practiced during the development of the lines had pushed gene frequencies in the different lines in the same direction. Hence, the progeny of inbred boars out of daughters of unrelated inbred boars would be genetically more homogeneous than their relationship would indicate.

Summary

Results are presented from an experiment in which inbred lines of swine were tested in linecross and topcross combinations in an experimental breeding herd and in which inbred and linecross boars were compared with outbred boars in two-sire farm herds. Data from 270 litters farrowed in 4 years were studied in the first part of the experiment. The farm topcross tests involved a total of 1648 litters in 93 herds over an 8-year period. The data from 228 litters in 14 of these herds were analyzed separately because the effect of topcrossing could not be separated from that of crossbreeding.

These results showed that linecrossing resulted in a recovery of the vigor lost during inbreeding, but showed no average advantage for the linecrosses over outbred controls. This latter is in contrast to results obtained in some other experiments which have shown linecrosses to be superior to outbred purebreds.

Topcrosses by inbred boars of predominantly Landrace breeding were significantly heavier at 5 months of age than non-topcrosses in the same herds. The actual difference was 11 lb. No other topcrosses by inbred boars showed any significant advantage in viability or rate of gain.

Topcross gilts by inbred boars were consistently superior to their controls in size and weight of litters raised to 5 months. Results from 27 herds showed significant increases for the topcross gilts of 1.0 pig and 156 lb. in these two traits, respectively.

There was an indication that inbred and linecross boars differed in their performance in topcrossing. Progeny of two-line cross boars showed a significant advantage in viability and quite large increases in pig and litter weights, whereas progeny of inbred boars of the parent lines showed no increase. In productivity comparisons, the gilts by inbred boars were superior to their controls, whereas gilts by linecross boars were not.

More general implications of these results and of the effects of the inbreeding and selection practiced in the development of the lines will be considered in a subsequent paper.

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EFFECTS OF INBREEDING, SELECTION, LINECROSSING AND TOPCROSSING IN SWINE. III. PREDICTING COMBINING ABILITY AND GENERAL CONCLUSIONS [1]

G. E. BRADFORD,[2] A. B. CHAPMAN AND R. H. GRUMMER [3]

University of Wisconsin, Madison

RESULTS of an experiment in which inbred lines of swine were developed and tested in several linecross and topcross combinations have been reported in two previous papers (Bradford *et al.*, 1958a; 1958b). These papers dealth with (1) the effects of inbreeding and selection practiced during the development of the lines and (2) differences between the different mating systems in which the inbred lines were tested. In the present paper, data will be presented on the predictability of performance from one system of mating to that in another and on the relative importance of general and specific combining ability and maternal effects as sources of variation in two-line cross data. In addition, the possible applications of inbreeding, linecrossing and topcrossing to the genetic improvement of swine will be discussed in the light of the results of the entire experiment.

Predicting Performance of Inbred Lines in Crosses

Data and Methods. Data were available on the performance of five lines as inbreds, in two-, three- and four-line crosses, and in topcrosses at the University farm, and on topcrosses from farms in the state. Data on a sixth line in certain of these mating systems were also available. These data, which have been published in the two papers referred to above, were used to study the predictability of results from one mating system to another.

The inbred line means were the combined averages from 3 years, 1948 to 1950, adjusted to zero difference in inbreeding between the lines. The linecrosses and topcrosses were produced in 1950, 1951 and 1952.

The line comparisons were made within year and age of dam subclasses by expressing each line mean as a percent of the overall mean for that subclass. The weighted average of the resulting percentages was then calculated and multiplied by the overall mean for the mating system to obtain the values used in the comparisons.

The ranking of the lines as inbreds was compared with their ranking as dam-lines in two-line crosses and as one of the parents of two-line cross

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[2] Present address: Department of Animal Husbandry, University of California, Davis, California.

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females on which productivity information was available. Each two-line cross female provided an observation for two lines. These three mating systems were compared for the four productivity traits: Number farrowed, number weaned, weaning weight of litters and average weaning weight of pigs.

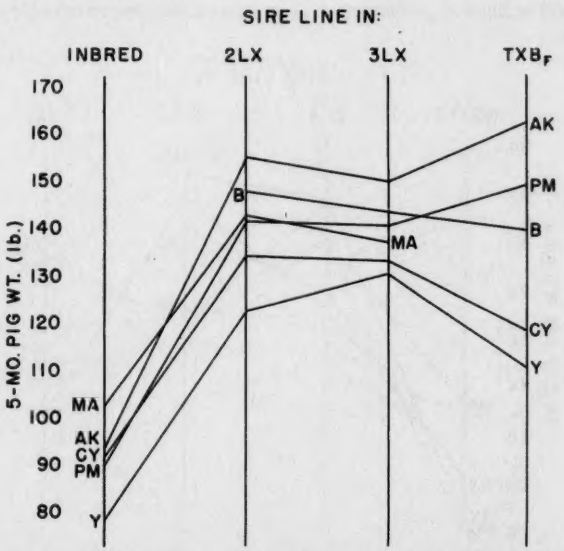

Figure 1. Ranking of the lines as inbreds and as sire lines in 2-line crosses, 3-line crosses and topcrosses for 5-month pig weight.

The lines were also compared as inbreds and as sire lines in two-line crosses, three-line crosses and topcrosses (University farm data) for 56-day weight and 5-month weight. The sire line means in the three types of crosses for 5-month weight were also compared with the ratios of the 5-month weight to the weaning weight of the inbreds.

Results. The comparisons of the lines for the three litter traits gave no evidence that the performance of a line as a dam-line of two-line crosses could be predicted from its performance as an inbred or that the average performance of all two-line cross females with this line as one parent could be predicted from either of the first two measures. This may be a reflection of the small numbers of observations in each group. For pig weaning weight there was fairly good agreement between the making of the lines as inbreds and as dam-line in two-line crosses. The means for all two-line cross females with a particular line in common did not differentiate the lines for this trait.

The ranking of the lines as inbreds and as sire lines in two- and three-line crosses (2LX and 3LX, respectively) and topcrosses (TXB_F) is shown in figure 1 for 154-day weight. As may be seen from the graph, the ranking of the lines was essentially the same in the three crosses but not the same for these as for the inbreds. The AK line, for instance, was the best line in combining ability but about average as an inbred, whereas the MA line, which had the highest performance as an inbred, was approximately average in crosses.

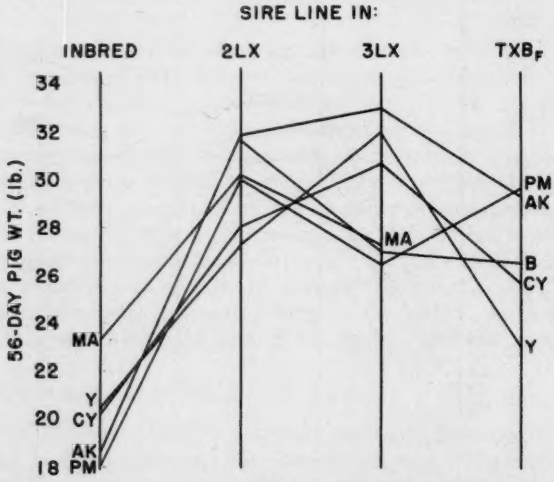

Figure 2. Ranking of the lines as inbreds and as sire lines in 2-line crosses, 3-line crosses and topcrosses for 56-day pig weight.

A comparison of the inbred line means for 56-day and 5-month weights in a previous study (Bradford *et al.*, 1958a) showed that as inbreds some lines consistently ranked higher for weaning weight than for 5-month weight. This suggested that the relationship of weaning weights with 5-month weights of the inbreds ought to be examined to determine if this might clarify the results shown in figure 1. The ranking of the inbred lines for 56-day weight is shown in figure 2. The ranking of the lines in the other three mating systems for this trait is also shown.

Figure 3 compares the ranking of the lines, as inbreds, for the ratio of 5-month weight to weaning weight, with their ranking as sire lines in two- and three-line crosses and in topcrosses, for 5-month weight. The results indicate that this ratio of 5-month weight of an inbred line predicts the combining value of a line for rate of gain more accurately than does the 5-month weight of the line alone.

These same lines were also used in the farm topcross tests reported by Bradford *et al.* (1958b). These results for 5-month weight failed to rank the lines in the same order as did the linecrosses and topcrosses at the University farm and therefore in the same order as the ratio of 5-month weight to weaning weight of the inbreds (figure 3). However, there was only a small number of boars per line in the farm topcross tests and the differences between the lines were small and non-significant. Therefore,

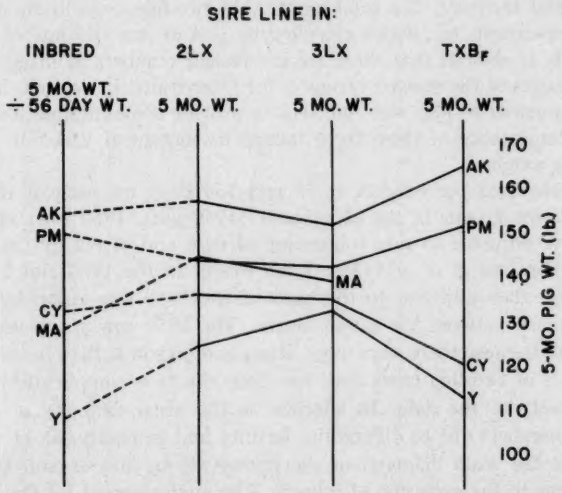

Figure 3. Ranking of the lines as sire lines in 2-line crosses, 3-line crosses and topcrosses for 5-month pig weight compared to the ratio of 5-month weight to 56-day weight of inbreds.

these results do not necessarily invalidate the predictive value of the measure of performance obtained in the experimental herd.

General and Specific Combining Ability and Maternal Effects

Knowledge of the relative importance of general and specific combining abilities and maternal effects as sources of variation in the progeny of inbred lines is also related to the question of methods of evaluating the lines and indicating the mating systems in which they should be used. If general combining ability accounts for most of the genetic variation, the lines may be used effectively in several different mating systems: two-line crosses, multiple-line crosses, or topcrosses. If specific combining ability is important, only certain crosses will be useful, and multiple-line crossing and topcrossing should be less satisfactory than two-line crossing.

Also, the lines will have to be tested in all possible combinations with each other before the best combinations can be found. If there are large differences between lines in their genetic potential for fertility or mothering ability, i.e., if maternal effects exist, it will be necessary to use certain lines only as female parents and others only as male parents. If the genetic basis of these maternal effects is such that they are transmitted to line-cross and topcross female progeny, the value of certain lines in these mating systems may be enhanced and that of others diminished.

Data and Methods. The total numbers of two-line cross litters and pigs in this experiment are shown classified by line of sire and line of dam in table 1. It is obvious that there are insufficient numbers of litters to provide estimates of the desired variances for litter traits. It was felt, however, that the number of pigs was sufficient to provide some information on the relative importance of these three factors as sources of variation in individual pig weights.

The individual pig weights at 56 and 154 days for each of the three two-line cross groups in the experiment (1950 gilts, 1950 sows and 1951 gilts) were adjusted to zero inbreeding of dam and of pig by the factors given by Braford *et al.* (1958a). Each weight in the 1950 and 1951 gilt groups was then adjusted to the basis of the 1950 sow group by adding the difference between the group means. The 1950 sow group was taken as the base because there were more litters and pigs in it than in the others.

Analysis of two-line cross data for these effects is complicated by non-orthogonality of the data. In addition to the usual difficulty of unequal subclass numbers due to differential fertility and mortality, all of the subclasses on the main diagonal of the line-of-sire by line-of-dam table are missing due to the exclusion of inbreds. The methods used for the analysis of these data take into account this non-orthogonality in the data.

To estimate the variance due to maternal effects, σ^2_m, and that due to additive gene effects in the pig, σ^2_s, the unweighted means of the cells, based on the unweighted means of the litters, were used. The following model was assumed.

x_{ij}=mean of cell with i^{th} line of sire and j^{th} line of dam

$=\mu+s_i+d_j+r_{ij}$

where: $i=1\rightarrow n$; $j=1\rightarrow n$; n=number of lines

μ=general mean

s_i=additive effect in pig of i^{th} line

$d_j=s_j+m_j$, and m_j=maternal effect of j^{th} line

r_{ij}=residual (interaction, litter effect and individual variation)

$\sum_{i=1}^{n} s_i=0$ $\qquad$ $\sum_{j=1}^{n} m_j=0$

$X_{i.}$=sum of i^{th} row of cell means

$X_{.j}$=sum of j^{th} column of cell means

TABLE 1. NUMBER OF TWO-LINE CROSS LITTERS FARROWED AND NUMBER OF PIGS RAISED TO 5 MONTHS, BY LINE OF SIRE AND LINE OF DAM

	Dam line[a]													
	MA		PM		AK		CY		Y		B		Sire line total	
Sire line[a]	No. litters	No. pigs	No. litters	No. pigs	No. litters	No. pigs	No. litters	No. pigs	No. litters	No. pigs	No. litters	No. pigs	No. litters	No. pigs
MA	—		3	9	2	6	2	14	1	0	1	7	9	36
PM	1	6	—		2	6	2	14	3	14	1	5	9	45
AK	1	5	3	8	—		2	8	2	7	2	8	10	36
CY	4	25	3	6	1	4	—		2	1	1	8	11	44
Y	2	8	3	15	3	14	2	11	—		2	7	12	55
B	1	8	2	10	1	5	3	14	2	7	—		9	44
Dam line total	9	52	14	48	9	35	11	61	10	29	7	35	60	260

[a] MA—Model A
PM—Purdue's Moderator } University of Wisconsin Chester White lines.
AK—Alfalfa King
B—Beltsville Chester White.
Y—Yorkshire—University of Wisconsin.
CY—Chester-Yorkshire line developed from a cross of PM and Y lines.

The following analysis of variance was then carried out:

Due to:	Degrees of freedom	Sums of squares	Mean square	Expected mean square
Fitting m_j	$n-1$	$\sum_{k=1}^{n} X_{.k}m_k$	S_1	$\cong \sigma^2_r+(n-1)\sigma^2_m$
Fitting s_i	$n-1$	$\sum_{k=1}^{n} (X_{k.}+X_{.k})s_k$	S_2	$\cong \sigma^2_r+2(n-2)\sigma^2_s$
Remainder	n^2-3n+1	By difference	S_3	$\cong \sigma^2_r$
Total	$n(n-1)-1$	$\sum_{i=1}^{n} \sum_{j\neq i} x_{ij}^2-\frac{X_{..}^2}{n(n-1)}$		

where $m_k=\dfrac{X_{.k}-X_{k.}}{n}$

$$s_k=\frac{(n-1)X_{k.}+X_{.k}-X_{..}}{n(n-2)}$$

The formula for s_k is the same as that given by Yates (1947) for estimating the effect of individual male parents in data from reciprocal crosses. The formula for m_k used here is not the same as that given by Yates for estimating the effect of female parents, since he assumed no maternal effects of the lines.

The variance due to interaction between line of sire and line of dam (specific combining ability), σ^2_{sd}, was estimated using σ^2_n and σ^2_e, between and within litter variances respectively, in conjunction with σ^2_r. σ^2_n and σ^2_e were estimated using weighted means. The detailed methods of estimation and the tests of significance used in these analyses are give by Bradford (1955).

Results. The constants s_k and m_k used in the calculation of the sums of squares for σ^2_s and σ^2_m may be looked upon as the additively genetic "effects" in the pig and maternal "effects" of the lines. They are presented in table 2.

TABLE 2. MATERNAL EFFECTS OF THE LINES (m_k) AND ADDITIVELY GENETIC LINE EFFECTS IN THE PIGS (s_k)

Line	Pig weaning weight		5-month pig weight	
	m_k	s_k	m_k	s_k
MA	.8	2.0	−1.4	5.1
PM	−1.4	−.5	−5.1	3.5
AK	−5.8	1.2	−14.9	9.7
CY	3.7	−3.5	2.0	−9.3
Y	.5	−1.1	14.2	−14.8
B	2.2	1.9	5.1	5.8

The results of the analysis of variance and the estimated variance components are given in table 3.

The values for m_k and s_k (table 2) indicate a negative association between the additively genetic effects in the pig and the maternal effects of the lines, although the number of lines is too small to determine whether this is a general phenomenon ($r=-.4$ for weaning weight and $r=-.8$ for 5-month weight). These two effects were assumed to be independent in

TABLE 3. ANALYSIS OF VARIANCE OF 2-LINE CROSS DATA FOR GENERAL AND SPECIFIC COMBINING ABILITY AND MATERNAL EFFECTS

A. Mean squares

Item	Pig weaning weight		5-month pig weight	
	Degrees of freedom	Mean squares	Degrees of freedom	Mean squares
Fitting m_j	5	43.7	5	186.4
Fitting s_i	5	25.0	5	456.8
Remainder	19	21.3	18	208.8
Between litters	19	116.8**	18	1270.4**
Within litters	235	35.9	212	454.2

B. Components of variance

Item	Pig weaning weight	5-month pig weight
σ^2_s General combining ability	.5	31.0
σ^2_m Maternal effect	4.5	—4.5
σ^2_{sd} Interaction (specific combining ability)	3.4	—8.1
σ^2_n Between litters	17.5	196.4
σ^2_e Individuals within litters	35.9	454.2

the model used to calculate σ^2_s and σ^2_m. Thus, the analysis of variance (table 3) may give a biased estimate of the relative importance of these two sources of variation. A negative correlation between the two would be expected to exaggerate s_i and minimize m_j.

The mean squares for s_i and m_j were not significant at the .05 probability level for either trait. The mean square for maternal effect of the line is larger than the additively genetic effect in the pig for weaning weight, whereas the reverse is true for 5-month weight. The possible bias in these results mentioned above should be kept in mind in their interpretation.

The F values for σ^2_{sd} were very close to unity for both traits, hence interaction between line of sire and line of dam was not indicated to be an important source of variation from these data.

Discussion. In order for inbreeding to be applicable to the genetic improvement of swine it must be possible to develop lines which can be maintained, and the performance of crosses of at least some of these lines

must be sufficiently superior to that of outbreds to pay for the cost of inbreeding.

The results from this and other experiments in the Regional Swine Breeding Laboratory indicate that it is possible to develop partially inbred lines which can be maintained, although the cost is quite high. Donald (1955) has reported results of an inbreeding experiment with the English Large Black which suggests the same conclusions. Some lines eliminate themselves due to the appearance of major abnormalities or to a severe reduction in vigor, some are culled because of poor performance in crosses, and the performance of those remaining is considerably lower than that of outbreds. Selection within lines during their development appears, in nearly all cases, to be ineffective in maintaining normal performance, and it may be that intense inbreeding in large numbers of small groups, without attention to within line selection, will lead to the development of more usable lines than mild inbreeding with selection.

Linecrossing appears to result in a recovery of the vigor lost through inbreeding, with three-line crosses superior to two-line crosses in traits affected by productivity of the dam. While the average of all linecrosses of a group of lines does not appear to exceed that of outbreds, it does appear possible to pick out certain lines which in crosses will have higher performance than outbreds. It does not seem possible to estimate from the information available whether the frequency of such lines is high enough and the performance of their crosses shows sufficient advantage to pay for the cost of developing and testing a large number of lines and maintaining those that are used in crossing.

Topcrossing, or the use of inbred or linecross males on outbred females, is a more practical method of using inbred lines than is linecrossing since it avoids the use of inbred females and the necessity of a producer purchasing females. Linecrossing would be preferred to topcrossing only if linecrosses were very markedly superior to topcrosses, and while the information on this is not as extensive as desired, there is no evidence that such is the case.

The results of topcrossing experiments indicate that certain lines can be selected which will give progeny superior to outbreds in rate of gain. Lines of Landrace breeding are in this category, although this may be due to superiority of the foundation stock or to genetic diversity rather than to the inbreeding and selection used in the development of the lines. A more important result of the topcrossing experiments is the finding that the gilts sired by inbred boars of all lines appear to have superior productivity compared to outbred gilts. The difference found in this experiment was sufficiently large to be of considerable economic significance.

The use of linecross boars in topcrosses does not appear to have given the same result as that of inbred boars. Progeny of two-line cross boars were superior in viability, rate of gain and total litter weight to their controls, but the topcross gilts by these boars were not different in

performance to outbred gilts. These results come from experiments with only one group of lines and their linecrosses, and further work is needed. Practically, two-line cross boars have the advantage over inbreds of being easier to produce and sell, although their use in a topcrossing program requires twice as many lines as does the use of inbred boars only. Three- or four-line cross boars have no demonstrated advantage over two-line crosses in topcrossing tests and there appears no reason for preferring them.

In a number of experiments on linecrossing and topcrossing reviewed, the effects of these mating systems have been confounded with crossbreeding. In practically all experiments, including the present one, where there have been direct comparisons of crossbreds and noncrossbreds, an advantage for the former has been found. Wherever it has been possible to separate crossbreeding effects from the effects of other mating systems (Whatley *et al.*, 1954; Bradford *et al.*, 1958b), it appears that the crossbreeding effect is added to the others. Thus where superior linecrosses or topcrosses have been produced in matings within breeds, linecrossbreds or topcrossbreds involving these lines have had the highest performance of all groups.

Methods of predicting the performance of lines in crosses from some measure of their performance as inbreds would remove the necessity of testing all available lines in crosses.

A method has been suggested for predicting the crossing performance of a line for rate of gain from a combination of the information on weaning and 5-month weights of the inbreds. Consideration of the weaning weight was indicated because of an apparent negative genetic correlation between maternal and additive effects in the pigs of the lines for rate of gain. It should be kept in mind that this method has been tested only on the data which suggested it, and will be useful in other data only if a negative association exists between maternal and additive effects in the pigs.

The relative importance of specific and general combining abilities and maternal effects is of course basic to the choice of methods of evaluating and using lines. In the analysis of the two-line cross data for these three sources of variation, the mean squares for general combining ability and maternal effects were not significant for either 5-month or 56-day weights. The test of significance used was subject to the limitation that the method of analysis assumed no correlation between these kinds of effects, whereas it appears that such did exist. The data on the inbreds and on the two-line crosses provide some evidence for this correlation.

Taking these variance estimates without regard to their statistical significance, it appears that maternal effects are relatively more important than general combining ability in weaning weights, with the reverse being true for 5-month weight, and that specific combining ability is not an important source of variation in individual pig weight. Henderson (1949) found specific combining ability accounted for a much larger proportion of the variation in litter traits than did either general combining ability or

maternal effects. Eaton *et al.* (1950), working with mice, found line differences in general combining ability for individual weights at 15 and 45 days but not for litter size or total litter weight. Maternal effects were important for individual weights but not litter weights, and specific combining ability was not evident for either kind of trait. These results do not permit general conclusions on these different sources of variation, except that it appears that specific combining ability is not important for rate of gain as estimated by weight for age.

The study of the relative importance of the different combining abilities in this experiment was based on lines of the Chester White and Yorkshire breeds. Other lines, such as the Landrace, which gave a different result in topcrossing, may give quite different results in studies of this kind.

The apparent negative genetic association between the additive effects in the pig and maternal effects of the lines in these data is in agreement with similar findings by other workers, e.g., Dickerson (1947) and Hazel (1954) working with swine, and Koch and Clark (1955) with beef cattle. This type of association offers one explanation for the ineffectiveness of selection for overall performance and therefore a reason why selection for cross performance is likely to result in more improvement than the more conventional selection within groups.

Summary

Data on six lines of inbred swine and on the two-, three- and four-line crosses between them and on the results of using these in topcrosses are compared for their predictability from one mating system to another. The following appeared to have predictive value: Pig weaning weight in inbreds with two-line crosses classified by dam-line; pig 5-month weight in two- and three-line crosses and topcrosses classified by sire-line with each other; and the ratio of 5-month to 56-day pig weights in inbreds with 5-month weight in two- and three-line crosses and topcrosses classified by sire-line.

Subdivision of variance in the two-line crosses for 56-day pig weights indicated that maternal effects are more important than general combining ability and that the opposite is true for 5-month pig weights. Neither showed evidence of specific combining ability. It was also indicated that there was a negative genetic correlation between the additive effects in the pig and the maternal effects of the line.

Possible applications of the results to the genetic improvement of swine are discussed.

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FEATHER MEAL AS A SOURCE OF PROTEIN FOR GROWING-FINISHING SWINE [1]

G. E. Combs, W. L. Alsmeyer and H. D. Wallace

Florida Agricultural Experiment Station [2]

FEATHER meal represents an available source of protein for animal feeds and while its use in poultry rations has been extensively studied, only a limited number of investigations have been conducted with swine.

Work by Thomas *et al.* (1955) indicated that feather meal was of no value for growing-finishing swine. The results of preliminary investigations conducted by Combs *et al.* (1956) showed that the inclusion of 5% of feather meal in a corn-soybean oil meal ration had no adverse effect on growth rate or feed conversion. Hall *et al.* (1957), using a corn-soybean oil meal-meat scrap type basal ration, found no significant difference in the daily gains of pigs fed rations in which one-third or two-thirds of the soybean protein was replaced with feather meal. Neither were any differences observed in the weight gains of pigs receiving steam-hydrolyzed feather meal as compared to feather meal hydrolyzed with lime.

The purpose of this investigation was to study the protein replacement value of various levels of feather meal (FM) fed to growing-finishing swine.

Experimental Procedure

Experiment I. Forty-eight weanling pigs of Duroc and Spotted Poland China breeding were randomly allotted, according to breed and initial weight, to six ration treatments. Each ration was fed to two groups of four pigs for a 91-day period. All pigs were fed and watered *ad libitum* in concrete pens. Live weights were recorded at two-week intervals and feed consumption records maintained.

The composition of rations fed is presented in table 1. All mixtures were calculated to contain approximately 16% crude protein. The FM used was a commercial steamed hydrolyzed meal that had been subjected to a maximum temperature of 250° F. and 35 lb. pressure for a 1.5 hour period.

Experiment II. Twenty-four weanling Duroc pigs were divided into four dietary groups, consisting of 6 pigs each, formed according to litter and weight. With the exception of ration 5, in which the lysine supplementation was increased to 0.46%, the rations fed are the same as rations 1, 2, 3

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[2] Department of Animal Husbandry and Nutrition, Gainesville. Acknowledgement is made to Wilson and Toomer Fertilizer Co. and The Dow Chemical Co. for supplying the feather meal and lysine used in this study.

and 5 shown in table 1. All pigs were individually fed in concrete pens for a 70-day period and weekly pig weights and feed consumption were recorded.

Results

A summary of average performance data and the analysis of variance for Experiments I and II is presented in table 2. The new multiple range

TABLE 1. COMPOSITION OF RATIONS USED

Ration number	1	2	3	4	5	6
Ration treatment	Basal	5% FM	7½% FM	7½% FM + Bovirum	7½% FM + DL-lysine	10% FM
Ground yellow corn, lb.	74.30	79.80	82.60	82.35	82.33	84.80
Soybean oil meal, lb.	21.00	10.30	4.80	4.80	4.80	.....
Feather meal, lb.	.....	5.00	7.50	7.50	7.50	10.00
Ground limestone, lb.	0.80	0.60	0.50	0.50	0.50	0.30
Steamed bone meal, lb.	1.30	1.70	2.00	2.00	2.00	2.30
Salt, lb.	.50	.50	.50	.50	.50	.50
Trace mineral mix, lb.[a]	.10	.10	.10	.10	.10	.10
Bovirum, lb.[b]				.25		
DL-lysine[c]						
Vitamin-antibiotic mix, lb.[d]	2.00	2.00	2.00	2.00	2.00	2.00

[a] Supplied the following in ppm.: Mn, 60; Fe, 73; Cu, 5; Co, 1.7; K, 7.8; and Zn, 25.
[b] Contains natural and cultured rumen bacteria; changed from .50 to .25 lb. at end of second week.
[c] Added at a rate of 0.27 and 0.46% in Experiments I and II, respectively.
[d] Contributed the following per lb. ration: vit. A, 2000 IU; vit. D, 198 IU; riboflavin, 8 mg.; calcium pantothenate, 8 mg.; niacin, 48 mg.; choline chloride, 208 mg.; folic acid, 0.5 mg.; vit. B_{12}, 10 mcg.; and chlortetracycline, 10 mg.

test as outlined by Duncan (1955) was used in determining the significance of treatment differences.

Growth Rate. In Experiment I pigs that received the 10% FM ration gained significantly slower than pigs fed any of the other ration treatments. The growth rate of the pigs on rations 1 and 2 was of the same magnitude and was significantly faster than that of pigs fed rations 3 and 4. While the growth response obtained from the addition of lysine (ration 5) was not sufficient to be significantly different from ration 3, the response was such that the gains of the pigs fed ration 5 were not significantly different from the fastest gaining groups (rations 1 and 2).

The growth data obtained from Experiment II are similar to those obtained from Experiment I. There were no significant differences between the daily gains of pigs fed the basal, 5% FM and 7½% FM plus lysine rations. The pigs receiving the unsupplemented 7½% FM ration gained at a significantly lower rate than pigs fed rations 1, 2 and 5.

Feed Data. Although there were no significant differences in feed intake among rations 1, 2, 3 and 5, it is apparent in Experiment I that all rations

TABLE 2. AVERAGE PERFORMANCE AND STATISTICAL DATA FOR PIGS ON EXPERIMENT I AND II

Ration no.	1		2		3		4		5		6	
Treatment	Basal		5% FM		7½% FM		7½% FM + Bovirum		7½% FM + DL-lysine		10% FM	
Experiment no.	I	II	I	II	I	II	I	II	I	II	I	II
No. of pigs	8	6	8	6	8	6	8	.....	8	6	8	.....
Av initial wt., lb.	42.0	41.3	42.5	40.6	42.3	40.6	41.6	.....	42.3	41.0	42.1	.....
Av. final wt., lb.	206.8	166.0	206.2	159.3	179.5	145.7	164.3	.....	189.5	157.3	116.3	.....
Av. daily gain, lb.	1.81**	1.78	1.80**	1.70	1.51	1.50[a]	1.35*	.....	1.62	1.66	0.76[a]	.....
Av. feed per lb. gain, lb.	3.53	3.43	3.42	3.36	3.76	3.53	3.60	.....	3.39	3.33	4.57**	.....
Av. daily feed intake, lb.	6.40**	6.11[b]	6.16**	5.69	5.67**	5.31	4.85*[a]	.....	5.48**	5.54	3.50	.....

Experiment I

Gain

** Significantly different from ration 3, 4 and 6 ($P<.01$)

* Significantly lower than ration 5 ($P<.05$)

[a] Significantly lower than all other rations ($P<.01$)

Feed efficiency

** Significantly higher than all other rations ($P<.01$)

Feed intake

** Significantly higher than ration 6 ($P<.01$)

* Significantly higher than ration 6 ($P<.05$)

[a] Significantly lower than rations 1 and 2 ($P<.05$)

Experiment II

Gain

[a] Significantly lower than all other rations ($P<.01$)

Feed intake

[b] Significantly higher than rations 2 and 5 ($P<.05$) and ration 3 ($P<.01$)

ANALYSIS OF VARIANCE OF GAIN, FEED EFFICIENCY AND INTAKE

Source	Experiment I					Experiment II			
	d.f.		M.S.			d.f.	M.S.		
	Gain	Feed	Gain	Eff.	Feed intake		Gain	Eff.	Feed intake
Treatment (T)	5	5	1.2157	.3912	2.2085	3	.0835	.0471	.6883
Replication (R)[a]	1	1	.0234	.0010	.0397	5	.0517	.0595	.7055
T × R	5	5	.0379	.0374	.1646	15	.0086	.0270	.1044
Error	36	..	.0382	.....	.....	..	.....	.....	.....

[a] Litters for Experiment II.

which contained feather meal were consumed in smaller quantities than the basal and that the extent of this decreased consumption was primarily influenced by the level of feather meal. At the 10% level of FM, feed consumption was significantly below all other treatments. As evidenced by the comparatively low consumption in lot 4, the presence of Bovirum also tended to render a 7½% FM ration unpalatable.

With the exception of the 10% FM ration which was significantly different from all other treatments, there were no significant treatment differences between the efficiency with which the Experiment I pigs converted feed to gain. However, a comparison of feed conversion figures, for rations which permitted comparable growth rates, indicates an improvement when 5% of FM was included and when the 7½% FM ration was supplemented with 0.27% DL-lysine.

Statistical analyses of feed consumption in Experiment II showed that the basal ration was consumed in significantly larger quantities than any of the rations containing FM. Although the level of FM did not significantly affect feed consumption, it is apparent that, as observed in Experiment I, the 5% FM ration was more readily consumed than the 7½% FM rations. The efficiency with which pigs on the various ration treatments converted feed to gain was not significantly different.

Discussion. Results reported here show that FM may be successfully incorporated into the ration of growing-finishing swine. The relatively low feed intake and consequent slow growth rate exhibited by pigs that received the 10% FM ration tends to exclude the possibility of FM constituting the sole source of supplementary protein. However, the performance of pigs fed rations containing 5% FM or 7½% FM plus lysine indicates that FM protein can be used to replace a portion (27–40%) of the protein present in a practical type corn-soybean oil meal ration.

Ration palatability rather than nutrient insufficiency appears to be the primary factor involved in the poor performance of the pigs fed the 10% FM ration. It became evident during the first week of Experiment I that the pigs were reluctant to consume this ration and, as evidenced by the daily feed intake figure, this reluctance continued throughout the experiment. A similar situation was observed with the 7½% FM ration supplemented with Bovirum. Even when the level of this material was reduced to 0.25% the ration was relatively unpalatable.

Since the calculated amino acid content of the FM rations indicated a deficiency of lysine, and feed consumption was not significantly different between the rations containing the low and medium levels of FM, nutrient imbalance rather than ration palatability appears to be the major factor which influenced the performance of pigs fed the unsupplemented 7½% FM ration. In Experiment I the addition of 0.27% DL-lysine to the 7½% FM ration brought the calculated lysine content up to the quantity present in the 5% FM ration and resulted in an improvement in rate and efficiency of gain when compared to the unsupplemented 7½% FM ration. When

the 7½% FM ration was supplemented with 0.46% DL-lysine (Experiment II) gains were significantly higher than on the corresponding unsupplemented ration. In both experiments there were no significant differences between the gains of the lysine supplemented pigs and those receiving the basal or the 5% FM rations, which indicates that at this level of FM, lysine supplementation was beneficial.

Summary

Two experiment involving 48 group-fed and 24 individually-fed weanling pigs were conducted to study the value of feather meal as a protein supplement for swine.

Three levels of feather meal were studied and the growth rate was found to be significantly influenced by the level fed. Pigs that received the ration containing 10% feather meal gained significantly slower than pigs fed any of the other rations.

Daily gains were significantly lower for pigs fed 7½% feather meal than for those fed the basal or 5% feather meal. Supplementation of the 7½% feather meal ration with 0.27% DL-lysine resulted in an improved rate of gain when compared to the unsupplemented ration (Experiment I); and, when the lysine supplement was increased to 0.46%, (Experiment II) daily gains were significantly increased.

The addition of Bovirum to the 7½% feather meal ration had an adverse effect on pig performance.

No significant differences were obtained among the growth rates of pigs fed the basal, 5% feather meal or 7½% feather meal plus lysine rations.

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FACTORS CONCERNED IN THE COMPARATIVE HEAT TOLERANCE OF JERSEY, HOLSTEIN AND RED SINDHI-HOLSTEIN (F_1) CATTLE

J. E. JOHNSTON, F. B. HAMBLIN AND G. T. SCHRADER

Louisiana Agricultural Experiment Station, Baton Rouge [1, 2]

IT is generally recognized that animals of Zebu breeding such as the Brahman or Red Sindhi are better adapted to hot environmental conditions than are animals of the European breeds. As a result of this greater heat tolerance, such animals have been used extensively in crossbreeding programs designed to develop cattle adapted to tropical areas. The physiological basis for this greater heat resistance among the Zebu breeds has not been clearly demonstrated although some possible explanations have been suggested. Kibler and Brody (1950) reported that Brahman cows had about 12% greater surface area per unit of body weight than Holstein and Jersey cows and suggested that the Brahmans may have had a lower basal metabolic rate. Other research at the Missouri station supported these observations and has been summarized by Worstell and Brody (1953). Unfortunately, the Missouri studies were conducted with Brahman cattle, which are poor milk producers. McDowell *et al.* (1954b) have compared the surface areas of Jersey and Red Sindhi-Jersey (F_1) cattle and found no breed difference. It has also been reported by McDowell *et al.* (1954a), that skin vaporization is no greater in Red Sindhi-Jersey cattle than in Jerseys. There does not appear to be any evidence to indicate that Zebu or Zebu crossbred cattle actually lose more heat than comparable animals of the European breeds. On the contrary, available information indicates that their greater heat tolerance is associated with lower heat production. These comparisons of heat balance mechanisms in Jersey, Holstein and Red Sindhi-Holstein cattle were undertaken in an effort to determine the basic reason for breed differences in heat tolerance.

Experimental

The data reported were collected during two separate studies. During both studies, respiratory data were collected with an open-circuit apparatus similar to that described by McDowell *et al.* (1953), except that the entire apparatus was heated to 105° F. to prevent condensation of moisture. Continuous samples of respired air were collected during each observation

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and analyzed for O_2, CO_2, CH_4 and N_2 with a laboratory model Burrell gas analysis apparatus. Water vapor content was estimated from temperatures of respired air determined with wet- and dry-bulb thermometer. Metabolic heat production estimates were based upon the assumption that each liter of O_2 consumed resulted in the production of 4.825 Kilo Calories of heat as proposed by Brody (1945). Surface areas were determined on each animal once during each study using a surface integrator (Brody, 1945). Rectal temperatures were determined using a 5-inch clinical thermometer.

Study 1. This study was conducted during July and August, 1955, with four animals each of Jersey, Holstein and Red Sindhi-Holstein (F_1) breeding. These 12 animals were all non-lactating and non-gestating. They were

TABLE 1. DIURNAL AMBIENT CONDITIONS WITHIN CLIMATIC CONTROL CHAMBER DURING EXPERIMENTAL PERIODS OF STUDY 2

	Hours											
	0800–1600			1600–2000			2000–0400			0400–0800		
Period	DBT[a]	VP[b]		DBT[a]	VP[b]		DBT[a]	VP[b]		DBT[a]	VP[b]	
Adjustment and 1	70	12	(64)	70	12	(64)	70	12	(64)	70	12	(64)
2	85	20	(70)	75	15	(68)	75	15	(68)	75	15	(68)
3	95	25	(60)	85	20	(66)	75	20	(92)	85	20	(66)

[a] DBT=Dry bulb temperature in ° F.
[b] VP=Humidity as mm. Hg. partial pressure of water vapor.
( )=Humidity as % saturation.

fasted for 12 hours prior to observations which were made before 9:00 a.m. in order to limit the effects of feeding and hot conditions on the measurements. Prior to observation, all animals grazed a mixed Dallis-Bermuda grass pasture and were fed a 16% crude protein concentrate ration at the rate of 0.5 lb. per 100 lb. of body weight daily. Respiratory observations were made on one animal of each breed group each day until all animals had been observed twice. The results of the two observations were averaged for analysis. Body weights were measured immediately following observations.

Study 2. This study was conducted during November and December, 1956, with three animals each of Jersey, Holstein and Red Sindhi-Holstein (F_1) breeding. The animals were selected so that similar ranges of milk production were represented in each breed group. They were placed in the L.S.U. Climatic Control Chamber and conditions adjusted as shown in table 1. An adjustment period of 10 days was employed to allow the animals to become familiar with the room, experimental manipulations and rations. Three 10-day experimental periods followed, the only change in management of the animals being temperature and humidity control.

In addition to the measurements taken during Study 1, skin temperatures were estimated at five points on the body of each animal with a 24-guage copper constantan thermocouple. Values were averaged to obtain a mean skin temperature. Serum protein-bound iodine (PBI) was esti-

mated on blood samples obtained the last day of each period by a modification of the procedure outlined by Moran (1952). Body weights were measured the last day of each period and the first two days of the subsequent period and averaged. Respiratory measurements were made twice on each animal during each period and the results averaged for analysis.

A 16% protein concentrate mixture was fed at the rate of 1 lb. per 4 lb. of 4% fat-corrected-milk (FCM) produced during the adjustment period. Alfalfa hay was fed during the night at the rate of 3 lb. per 100 lb. of body weight. A high quality corn—soybean silage was fed *ad libitum* during the day. Continuous records of dry matter intake were maintained. Cows were milked twice daily at 12-hour intervals and an aliquot sample of each milking preserved for fat test at the end of each period.

Results and Discussion

Study 1. The results of this study are shown in table 2. While there were variations among animals within breed with respect to estimated

TABLE 2. RESPIRATORY ACTIVITY, PHYSICAL CHARACTERISTICS AND HEAT PRODUCTION OF BREED GROUPS DURING STUDY 1

Breed	Jersey	Holstein	Red Sindhi-Holstein
Body temperature, ° F.	101.0	101.2	101.0
Respiration rate, per min.	30	35	30
Respiratory minute volume, L.	54	93	48
Oxygen consumption, L./hr.	59	100	52
Heat production, K. Cal./hr.	284	482	266
Body weight, Kg.	406	582	534
Surface area, M^2	3.81	4.82	4.67
Surface area per unit of body wt., M^2/100 Kg.	0.94	0.84	0.88
Heat per unit of body wt., Cal./100 Kg.	70	84	50
Heat per unit of surface area, Cal./M^2	75	100	57

heat production, the Red Sindhi-Holstein crossbreds exhibited significantly ($P<.05$) lower values per unit of body weight, or per unit of surface area than animals of the other breeds. This becomes extremely important under thermal stress since most of the body heat must be lost via the skin under such conditions.

There appeared to be little relationship among respiration rates, respiratory minute volume and oxygen consumption. This lack of relationship suggests that respiratory rate is of little value as a measure of the rate of heat loss through respiratory vaporization or as an index of the loss of alkaline reserve during thermal hyperpnea.

Study 2. The mean body weight, surface area, forage dry matter consumption and concentrate dry matter consumption of the animals in this study are shown by breed and period in table 3. It may be noted that the animals in this study weighed less than those used in Study 1 but

their surface areas were quite comparable except for the Red Sindhi-Holstein crossbreds. This might be expected since the animals used in Study 1 were dry and those in Study 2 were lactating. The changes in milk production during the study were not marked and indicated little effect of hot conditions. This may have been a carryover of summer adaptation. The failure of the Red Sindhi-Holstein crossbreds to show a decline in FCM production during the study was due to one animal which had been fresh only 4 weeks at the beginning of the study and increased her production.

Heat production, body temperature, respiratory activities, and serum protein-bound iodine values are summarized in table 4. The Red Sindhi-

TABLE 3. BODY CHARACTERISTICS, FCM PRODUCTION, FEED DRY MATTER INTAKES AND PROTEIN-BOUND IODINE BY BREED FOR EACH PERIOD

Period	Breed	Body weight (Kg.)	Surface area (M^2)	FCM (lb./day)	Forage intake (lb./100 Kg./day)	Concentrate intake (lb./day)	Serum PBI (mcg./ 100 ml.)
1	Jersey	398	3.80	27.8	4.1	7.1	2.44
2	Jersey	392	3.80	26.5	4.6	7.1	2.87
3	Jersey	382	3.80	24.2	4.0	7.1	1.86
1	Holstein	490	4.81	27.0	3.8	6.8	2.21
2	Holstein	483	4.81	27.6	4.6	6.8	2.90
3	Holstein	470	4.81	25.8	3.5	6.8	1.76
1	R.S.-Hol.	440	4.30	32.0	3.8	6.8	1.91
2	R.S.-Hol.	438	4.30	33.5	4.2	6.8	2.00
3	R.S.-Hol.	430	4.30	32.7	4.2	6.8	1.75

Holstein cross breds produced significantly ($P<.05$) less heat per unit of body weight or per unit of surface area than the Holsteins during all periods and less than the Jerseys during all but period 1. The change in heat production of the European breeds during the study is of particular interest since it would appear to indicate something about the process of adaptation. These animals showed a consistent rise in heat production from period 1 to period 2. This change would appear to be the result of increased metabolism associated with elevated body temperatures and respiration rates and was followed during period 3 with a decline in heat production even though thermal stress was increased. The decline during period 3 would appear to be associated with the adaptive mechanisms of the body and was exhibited by all animals even though the crossbreds had not shown a marked rise during period 2. The possibility of thyroid involvement is indicated by the fact that PBI values showed similar trends. It should be noted that forage dry matter consumption also rose during period 2, explaining the source of energy for the increased heat production. While there was a tendency for milk production of cows of the European breeds to decline during the study, these changes would not appear to explain the changes noted in heat production.

TABLE 4. HEAT PRODUCTION, BODY TEMPERATURES AND RESPIRATORY ACTIVITY OF LACTATING COWS EXPOSED TO VARYING DEGREES OF THERMAL STRESS

Period	Max. temp. (° F.)	Breed	Heat prod. total (K-Cal./hr.)	Heat prod. per 100 Kg. (K-Cal./hr.)	Heat prod. per M^2 (K-Cal./hr.)	Rect. temp. (° F.)	Skin temp. (° F.)	Resp. rate (per. min.)	Resp. vol. (L./min.)	Resp. vaporization (K-Cal./hr.)
1	70	Jersey	568	142	149	102.0	91.5	37	67	43
2	85	Jersey	731	186	192	102.4	95.1	73	145	100
3	95	Jersey	557	146	147	104.4	99.4	77	181	135
1	70	Holstein	834	170	174	101.9	93.1	40	94	65
2	85	Holstein	876	181	182	102.9	95.6	70	149	110
3	95	Holstein	635	134	132	104.7	98.9	63	168	124
1	70	R.S.-Hol.	572	130	133	101.6	90.9	32	58	34
2	85	R.S.-Hol.	564	129	131	101.7	95.1	40	81	62
3	95	R.S.-Hol.	491	114	114	102.1	98.9	40	95	77

Respiratory activities and body temperatures showed marked response to increasing thermal stress. Again, as in Study 1, respiration rate did not provide an adequate index of change in minute volume. The rise in respiratory minute volume and vaporization from period 2 to period 3 was associated principally with changes in tidal volume rather than respiration rate. The importance of the skin as an organ of heat loss is emphasized by the small portion of the total heat lost by means of respiratory vaporization. This ranged from 16 to 24% of total metabolic heat in the three breeds during period 3. The similarity of skin temperatures in animals of the three breeds during each period suggests that the rate of heat loss per unit of skin surface area was similar in magnitude.

The superior heat tolerance of the Red Sindhi-Holstein crossbreds over animals of the European breeds is demonstrated by their lower body temperatures and respiration rates under thermal stress. This would appear to be due to lower total heat production and heat production per unit of surface area. The evidence in these data supports the theory that the greater heat tolerance of Indian type cattle is due to lower basal heat production rather than a more efficient heat loss mechanism. When the data from period 1 of Study 2 are adjusted for resting heat production estimated in Study 1, the average heat increment associated with each additional pound of FCM produced is 10, 13 and 10 Kilo Calories per hour for Jerseys, Holsteins and Red Sindhi-Holsteins, respectively. While these are rough approximations based on small numbers of animals and few observations, the concept is extremely important to breeders attempting to develop cattle adapted to hot climates. High producing strains of tropically adapated species of cattle would be under nearly as much thermal stress as similar animals of European breeds due to the lessened importance of basal heat production relative to the heat increment associated with milk production. Admittedly, other factors such as skin and coat color, ruminal heat production and individual variations in skin vaporization, may be involved in the development of tropically adapted cattle; however, the relative importance of such factors has not been established in lactating animals.

There is a need for more extensive studies of the basic factors associated with heat tolerance of lactating cattle. Such studies should include heat tolerant strains such as the Zebu as well as the European breeds of dairy cattle in an effort to determine whether there are sufficient variations in heat production and heat loss mechanisms to warrant selection for such factors within the European breeds.

Summary and Conclusions

Two studies were conducted during which lactating and non-lactating Jersey, Holstein, and Red Sindhi-Holstein (F_1) cattle were compared under varying conditions of thermal stress. The total heat production and

heat production per unit of surface area of non-lactating Red Sindhi-Holstein crossbreds were shown to be lower than those of Jerseys and Holsteins. The principal difference in heat balance between lactating Red Sindhi-Holsteins and the European breeds was in heat production rather than heat loss. The difference in heat production appeared to be associated with basal heat production rather than with that due to milk production. The increment of heat associated with each pound of FCM produced was estimated to be 10, 13 and 10 Kilo Calories per hour for Jerseys, Holsteins and Red Sindhi-Holstein crossbreds, respectively.

Exposure to elevated temperatures and humidities resulted in initial increases in heat production apparently due to elevated body temperatures and respiratory activity among the Jersey and Holstein cows. Red Sindhi-Holsteins did not show a marked initial rise in heat production or body temperature. All animals showed a decrease in heat production apparently due to adaptation during the last period of the study even though air temperature and humidities were elevated again. Serum protein-bound iodine levels paralleled changes in heat production suggesting that the thyroid gland may have been involved in adaptation.

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A COMPARISON OF URINARY AND BLOOD SERUM CREATININE WITH LIVE PROBE AS MEASURES OF LEANNESS FOR LIVE SWINE [1]

R. L. Saffle, L. E. Orme, D. D. Sutton, D. E. Ullrey and A. M. Pearson [2]

Michigan Agricultural Experiment Station [3]

THE increased interest in production of leaner pork has focused attention upon the importance of developing methods for predicting leanness in live hogs. Brody (1945) showed that in general urinary creatinine excretion was related to lean tissues of the animal body. Dinning *et al.* (1949), in a study of beef steers, reported that protein intake did not affect creatinine excretion, which was relatively constant from day to day. Friedemann *et al.* (1947) found in human subjects that creatinine excretion varied somewhat from week to weeek, independent of the small change of body weight. However, the maximum variation of the creatinine coefficient (milligrams of urinary creatinine excreted in 24 hours per kg. of body weight) was in the order of $\pm 5\%$ of each mean for all subjects during a 9-week period. Lofgreen and Garrett (1954) in a study of beef steers found a significant correlation between urinary creatinine coefficient and separable lean in the soft tissue of the 9-10-11th rib cut ($r = .67$). Miller and Blyth (1952) found that for humans, the lean body mass agrees within $\pm 13\%$ of the densiometrically measured value in 90% of the cases, while for large groups ($N = 50$ or more) agreement was within $\pm 2.0\%$. Wurthier and Stratton (1957) in a study of beef cattle found a significant correlation between blood serum levels of creatinine and the percentage of lean in the boned 9-10-11th rib cut ($r = .55$) and rib eye area corrected for carcass weight ($r = .52$).

This study was designed to determine if either creatinine coefficient or blood serum creatinine could be advantageously used to predict leanness of live hogs. In addition, a comparison was made between live probe and the two creatinine measurements to see which was the more sensitive in detecting leanness.

Materials and Methods

Thirty-five barrows ranging in slaughter weight from 180 to 215 lb. were used in this study. They consisted of 24 Yorkshires, 5 Durocs, 1 Hampshire, 4 crossbred Chester White X Duroc, and 1 Chester White. Each hog was

[1] Journal Article 2187 Michigan Agricultural Experiment Station, East Lansing.

[2] Department of Animal Husbandry.

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placed in a metabolism cage for a five-day period. Urine was collected and measured daily and aliquots were analyzed for creatinine by the method described by Clark and Thompson (1949). The creatinine coefficient was calculated for each day and the daily values averaged together. Blood samples were taken immediately before and after the five-day collection

TABLE 1. CORRELATION COEFFICIENTS FOR THE CREATININE COEFFICIENT, LIVE PROBE AND BACKFAT THICKNESS WITH OTHER CARCASS MEASURES

Item	Blood serum creatinine	Creatinine coefficient	Live probe
Creatinine coefficient	.27	...	—.61
Live probe	—.29	—.61	...
Lean cuts-live basis	.37	.58	—.80
Lean cuts-carcass basis	.33	.65	.88
Primal cuts-live basis	.40	.52	—.70
Primal cuts-carcass basis	.39	.64	—.86
Percent fat trim	—.44	—.54	.60
Lean area-10th rib	.35	.55	—.69
Lean area-last rib	.35	.66	—.75
Specific gravity-ham	.08	.41	—.74
Backfat thickness	—.22	—.65	.90
Dressing percentage	.06	—.46	.55
Carcass length	.12	.50	—.65
Composition of ham			
Percent fat	—.21	—.58	.73
Percent water	.24	.65	—.82
Percent protein	.29	.63	—.81
Combined fat, water, protein	.33	.65	.83

period and were analyzed for creatinine by the alkaline picrate method as described by Reiner (1953).

Live probes were made by the method of Hazel and Kline (1952). The hogs were then handled, slaughtered, cut and measured in the same manner as reported by Pearson *et al.* (1956). Specific gravity of the right untrimmed ham was determined by the under-water weighing technique described by Brown *et al.* (1951) with variation as discussed by Pearson *et al.* (1956). Since Price *et al.* (1957) found that the specific gravity of the untrimmed ham was a good indication of the lean of the entire carcass, the entire right ham was boned and ground. Chemical analyses were made for moisture, ether extract and protein according to procedures outlined by Benne *et al.* (1956).

Results and Discussion

Examination of the correlations between creatinine coefficients and various measurements of carcass leanness (table 1) revealed that the creatinine coefficient was negatively associated with the amount of fat

in each case. Blood serum creatinine followed the same general trend as the creatinine coefficient. In each instance, the relationships between the blood serum creatinine and the various measurements of leanness were smaller than the association for creatinine coefficient and the corresponding measurement. The lower values for the blood serum creatinine may have been due to the very small amount of creatinine in the blood at any one time. Furthermore, the blood serum creatinine was only an average of two samples, before and after the collection period, whereas the creatinine coefficient was based on the average amount of creatinine excreted in

TABLE 2. MULTIPLE CORRELATION COEFFICIENTS FOR CREATININE COEFFICIENT AND LIVE PROBE WITH OTHER CARCASS MEASUREMENTS

Item	Multiple correlation coefficients for creatinine coefficient and live probe
Lean cuts-live basis	.91
Lean cuts-carcass basis	.89
Primal cuts-live basis	.71
Primal cuts-carcass basis	.87
Percent fat trim	.64
Lean area-10th rib	.71
Lean area-last rib	.79
Specific gravity-ham	.74
Backfat thickness	.91
Dressing percentage	.57
Carcass length	.66
Composition of ham	
Percent fat	.75
Percent water	.84
Percent protein	.82

the urine over a 5-day period. Thus, it is conceivable that more frequent bleedings may have resulted in improvement of the correlation between blood creatinine and other measures of leanness.

The highest relationship of the creatinine coefficient with the various measurements of leanness was with the loin lean area of the last rib, which had a correlation coefficient of 0.66. Correlations of approximately the same magnitude were found with the backfat thickness, lean cuts-carcass basis, primal cuts-carcass basis, and the combined fat, water, protein content of the ham. This confirmed the work of Brody (1945) and the studies on beef by Lofgreen and Garrett (1954) showing that the creatinine coefficient is correlated with lean body mass. The correlation coefficient of the blood serum creatinine had the same general trend as urinary creatinine but a lower relationship in each case. The highest correlations were found with the percent fat trim (—.44) and primal cuts-live basis (.40).

In order to determine if creatinine was measuring leanness undetectable by the live probe, the relationship between the live probe

and the various measurements of leanness was calculated. In each case, this relationship was higher than the correlation of either urinary or blood creatinine with the corresponding measure (table 1). It must be concluded, therefore, that live probe is superior, since it is not only more closely related to other measures of leanness but is quicker and simpler to obtain than either of the creatinine values.

In order to test whether the live probe and creatinine coefficient could be used in combination to predict leanness more accurately than either method alone, a multiple correlation of the creatinine coefficient and live probe was determined against the various other carcass measurements (table 2). However, the multiple correlation was about the same as the live probe and did not improve the predictive value sufficiently to justify the added work.

Summary

Blood serum creatinine and creatinine coefficients were determined for 35 barrows. The creatinine coefficients and blood creatinine values were correlated with live probe and other measurements of carcass leanness. The results indicate that creatinine may be used to predict the amount of lean in the live hog. The urinary creatinine coefficient was more highly correlated with other measures of leanness than blood creatinine, but in general was not as high as the live probe. Therefore, there would be no major advantage in using creatinine coefficient as a measure of leanness since the live probe was simpler to obtain and was more closely correlated with leanness.

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NEWS AND NOTES

Nineteen Hundred and Fifty-Eight Annual Meeting. The annual meeting will be held at the Hotel Sherman, Chicago, on November 28 and 29. You will be sent a questionnaire in early June regarding papers, and titles are to be submitted to the Secretary's office by July 20. Abstracts of papers are to be submitted by August 15. Only members of the Society can submit papers, but associate authors can be non-members.

Nominations for the Morrison Award. All nominations are due in the Secretary's office by June 15. The rules governing this award were amended in 1952 and are as stated in J. Animal Sci., Vol. 11, No. 4, pp. 807–809. All nominations must be accompanied by a brief biography of the nominee, a list and reprints of publications concerning the research, and a statement of the importance of this research to the livestock industry and animal science. The cash award will be $1,500 and a gold watch.

Nominations for the American Feed Manufacturers' Award. The rules governing this award are published in the J. Animal Sci., Vol. 7, No. 2, pp. 257–258, 1948. Two changes have been made in the rules to include (1) research work published during the three years immediately preceding the year of recognition (this has formerly been a two-year period) and (2) that the recipient must be affiliated with a public institution, hospital or similar organization at the time of selection.

Nominations for this award should be sent to the Secretary's office by June 15. The purpose of this award is to recognize an individual conducting outstanding original research in the fields of beef cattle, horse, sheep and/or swine nutrition. The cash award is a check for $1,000 presented by the American Feed Manufacturers' Association.

The annual meeting of Western Section of the American Society of Animal Production will be held July 14, 15 and 16 at the University of California, Davis.

In an announcement in the February issue relating to the next annual meeting of European Association of Animal Production, the address of the Secretary of that Association was incorrectly given. It should read: Mr. K. Kallay, Secretary-General, Federation Europeene De Zootechnie, via Quintino Sella 54, Rome, Italy.

The American Society of Animal Production was represented along with other national societies and organizations at the Centennial Celebration of the establishment of Iowa State College. H. H. Stonaker, Secretary-Treasurer, represented the Society in the absence of Dr. W. M. Beeson, who was recovering from an automobile accident.

The Third World Congress on Fertility and Sterility will be held June 7 to 13, 1959, in Amsterdam, Holland. The program will include sections on female sterility, male sterility, basic research and animal reproduction, and psycho-sexual problems. Title for papers should be sent not later than June 1958 to both Dr. Alfonso Alvarez-Bravo, Avenida Horacio 1506, Mexico City 5, D. F., Mexico, chairman, and Prof. B. S. ten Berge, Academisch Ziekenhuis, Groningen, Holland, executive member of the Program Committee.

A. E. Darlow, Dean of the College of Agriculture and Vice President of Oklahoma State University, has been chosen as the honor guest of the American Society of Animal Production at its meeting November 28 and 29, 1958. Dr. Darlow's portrait will be presented to the Saddle and Sirloin Club at the time of the banquet in his honor Sunday evening, November 30.

Roy M. Kottman, Associate Dean of Agriculture and Professor of Animal Husbandry at Iowa State College, has resigned effective June 30 to accept appointment as Dean and Director of the College of Agriculture, Forestry, and Home Economics, and Director of the Agricultural Experiment Station at the University of West Virginia. A native of Iowa, he received his B.S. and Ph.D. degrees at Iowa State College and M.S. degree from the University of Wisconsin.

L. M. Winters died recently while serving as adviser on animal breeding problems in Iran, under the sponsorship of the U. S. International Cooperation Administration. He was Professor of Animal Breeding at the University of Minnesota until his retirement July 1, 1956.

Thomas H. Bartilson, who was Assistant Chief of the Animal Husbandry and Poultry Division prior to its reorganization in 1957, now carries the title of Assistant Director, Animal Husbandry Research Division.

R. T. Clark is on leave from his regular duties as regional coordinator for the western regional project on beef cattle breeding to participate in the livestock improvement program in Argentina, South America, sponsored by the Food and Agriculture Organization of the United Nations. He is stationed in Buenos Aires. C. E. Shelby is acting regional coordinator for W-1 during Dr. Clark's absence.

A. D. Pratt, Associate Professor of Dairy Science at the Ohio Agricultural Experiment Station, is on a one-year leave of absence to serve as Animal Nutritionist in FAO's livestock improvement program in Argentina, S. A. He is stationed in Buenos Aires.

Estel H. Cobb, former Assistant Professor in the Animal Husbandry Department at New Mexeco A. and M. College, joined the Animal Science staff of the Hawaii Agricultural Experiment Station in January. He will have leadership of Hawaii's contributing project to W-1, the western regional project on beef cattle breeding.

L. E. Card, Head of the Department of Animal Science, University of Illinois, is serving as group leader of the University's technical aid team in North Central India. During his absence, Dr. H. M. Scott, Professor of Poultry Husbandry, is Acting Head.

Jacob W. White was appointed Extension Sheep Specialist in Georgia, effective January 1, 1958, with headquarters at Athens. He received his B.S. degree in Animal Husbandry at Oklahoma State University in 1951.

Morris L. Hemstrom was appointed Assistant Extension Animal Husbandman in Nebraska, February 1, 1958, with headquarters at Lincoln. He received his B.S. degree at Colorado State University in 1950.

Dewey Johnson who has recently completed requirements for the Ph.D. degree in Poultry Nutrition at Rutgers University has been named an associate in Animal Nutrition research at the Lime Crest Laboratory of Limestone Products Corporation of America, Newton, New Jersey.

Clarence F. Winchester, Animal Physiologist at the USDA's Agricultural Research Center, Beltsville, Md., has accepted a 2-year appointment by the International Cooperation Administration as Livestock Advisor to the Government of Ceylon. He will be stationed at Ceylon University at Peradeniya.

S. A. Ewing will join the Animal Husbandry staff at Iowa State College as Assistant Professor in charge of beef cattle teaching and management research on June 16, 1958. Mr. Ewing is a native of Oklahoma, holds B.S. and M.S. degrees in Animal Husbandry and Nutrition from Oklahoma State University and will complete his Ph.D. degree in Animal Nutrition at the same institution in June, 1958.

J. K. Loosli is spending a six-month sabbatical leave at the Department of Animal Husbandry and Nutrition of the University of Florida and will return to Cornell University in August 1958.

Donald L. Staheli, who has been serving with the U. S. Air Force since completion of his Ph.D. degree in Animal Science at the University of Illinois in 1956, has joined the Research Laboratories of Swift and Company, Chicago, Illinois.

E. M. Harmon will have completed in October five years of service as Senior Livestock Advisor to the Iranian Government under the Point-4 program. His accomplishments in this capacity have won for him a letter of commendation from Clark S. Gregory, Director, USOM/Iran.

Professor Frank B. Morrison, distinguished educator and author and Professor Emeritus of Animal Husbandry and Nutrition, Cornell University, died suddenly of a coronary attack on Monday, April 7, in an Atlantic, Iowa, hospital. Professor and Mrs. Morrison were enroute from Stillwater, Oklahoma, to the home of their son at Clinton, Iowa, when he was stricken.

Literature of Agricultural Research by J. Richard Blanchard and Harald Ostvold; 231 pages; $5.00; University of California Press, Berkeley, 1958. The first of a series of bibliographic guides sponsored by the University of California libraries. This guide to information and literature is divided into sections, agriculture in general, plant sciences, animal sciences, physical sciences, food and nutrition and social sciences. Under animal sciences, the reference works under animal husbandry are arranged in the following categories: abstracting journals, bibliographies and indexes, encyclopedic works, dictionaries, directories, handbooks, texts, etc., history: general, history: cattle: individual breeds, history: sheep, history: swine, history: horses, geography, periodical lists, miscellaneous. The book should be particularly useful to writers and certain investigators searching references to the literature outside their own field of specialization.

Nutrition Animale, Vol. 1, Donees Generales sur la Nutrition et l'Alimentation by R. Jacquot, H. Le Bars and H. Simonnet; 472 pages; J.-B. Bailliere et Fils, Editeurs; 19 Rue Hautefeuille, Paris, 1958. According to the general plan described in the front of the volume, this is one of a 9-volume series published under the general title of Nouvelle Encyclopedie Agricole. The subject matter is treated in a descriptive manner under such classifications as carbohydrates, fat, protein, minerals, vitamins, and their digestion in the different classes of farm animals. There are numerous figures, photographs and charts to illustrate differences between herbivores and omnivores, for example, in digestion processes. This volume is paper-bound.

Notice has been received of publication of the 3rd edition of Introductory Animal Husbandry by Arthur L. Anderson, and the revised edition of Modern Breeds of Livestock by Hilton M. Briggs. The importance of these books in Animal Husbandry is indicated by the number of adoptions by colleges in the United States. Both books are published by the Macmillan Company, New York.

Charles M. Thompson, formerly Director of Research and Nutrition of Colorado Milling Company, and recently engaged in the feed consulting business, died April 7,

1958. Dr. Thompson held B.S. and M.S. degrees from the University of Kentucky and a Ph.D. from Oklahoma State University.

William E. Townsend who recently received his Ph.D. degree in Animal Husbandry at Michigan State University has joined the staff of the research laboratories of Swift and Company.

Hilton M. Briggs has been named President of South Dakota State College at Brookings. Prior to his recent appointment, Dr. Briggs served as Dean of the College of Agriculture and Director of the Agricultural Experiment Station, University of Wyoming, Laramie.

Ralston Purina Research Fellowships. Winners of these fellowships have been announced by the Ralston Purina Company after a meeting of the Awards Committee in St. Louis, March 24. Recipients and their college or university of undergraduate study are listed according to subject matter interest as follows:

Animal Husbandry. Robert F. Hendrickson, Wisconsin and Oklahoma State; Allen H. Trenkle, Nebraska and Iowa State; J. Elton Zimmerman, Kansas State and Oklahoma State.

Dairy Husbandry. Marion M. Anderson, University of Iowa and Missouri; John W. Suttie, Wisconsin; Harold H. Van Horn Jr., Kansas State.

Poultry Husbandry. William R. Featherston, Arkansas; Bruce E. McDonald, University of Alberta; Robert M. O'Neil, Missouri.

Veterinary Medicine. Wilfred L. Collette, University of Florida and Alabama Poly. Inst.

This is the tenth year of the program. Each award is for $1800 on an annual basis. The Committee evaluates the applicants on the basis of scholastic record, proposed research project, recommendations and other pertinent data included on the application form.

INFORMATION FOR CONTRIBUTORS

1. Papers may be submitted reporting work whose ultimate objective is clearly the solution of a livestock production problem and whose contents should be of interest particularly to those dealing with livestock production problems. Review papers on subjects of general interest will also be published at intervals, and will be prepared on invitation by the Editorial Board.

2. All papers will be subject to critical review by the Editorial Board, or others designated by the Editor. Papers needing revision will be returned to authors and should be revised and returned promptly. Papers not suitable for publication will be returned to authors with a statement of reasons for not accepting them.

3. Papers submitted should be based on new and adequate information. The following are guides to style and form:

   (a) Manuscripts must be typed, double-spaced, and the ribbon copy submitted.

   (b) Pages must be numbered. The lines of text must be numbered in the left margin, beginning with one at the top of each page. This does not apply to tabular material.

   (c) Tables should be as few and as simple as is feasible for presentation of the essential data.

   (d) Legend for each figure should be typed on a separate sheet. Photographs should be carefully prepared and labeled. Magnifications of photomicrographs should be indicated on legends. Charts should be prepared in India ink. Typed material on charts is not acceptable. Material that will not reproduce satisfactorily will be returned for revision. For suggestions on preparing illustrative material see the "Style Brief" published by the Wistar Institute Press, Philadelphia. If in doubt, consult the Editor before putting material in finished form.

   (e) The introduction should consist only of a paragraph indicating very briefly the nature of the question asked and the reason for asking it. Reference to relevant literature should be made later in the discussion of the results. Concise presentation of data is desired and there should be evidence of statistical examination wherever obviously useful in the interpretation. Unsupported hypotheses should be avoided. The organization of a manuscript may vary with the nature of the material, but all manuscripts should contain a clear statement of application of the results or their relation to some problem of the livestock industry. Each paper should end with a brief summary in the form of an abstract containing the most pertinent data in the briefest possible form.

   (f) Literature citations must be listed in alphabetical order and should be referred to in the text by author and date, i.e., Castle (1924), or (Castle, 1924). Citations may be referred to by number in review articles where the number of citations is too great to permit convenient use of the above form. "Literature cited" lists should be typed in a style used in a current issue of the JOURNAL.

4. Manuscripts will be edited in the order received, and accepted papers will be published as nearly in this order as feasible.

5. There will be a charge for making engravings in excess of one page per paper and of printing tables in excess of two pages per paper. The author will be asked when his manuscript is accepted to indicate the institution, agency or individual that will assume responsibility for the charges and for the reprints ordered at that time.

6. Authors will receive galley proof of articles together with a form for supplying an abstract for "Biological Abstracts." *Corrected proof and abstract should be mailed to the Editor within three days after they are received. Failure to do this may result in delay of publication until the next issue.*

7. One author of each manuscript should be a member of the Society. Papers by non-members can be published only after approval by the Executive Committee.

8. A "News and Notes" section will be published in each issue, including Society announcements, changes in positions and other items of professional interest to members. All such items should be sent promptly to the Editor.

9. Abstracts of papers to be presented at the annual meetings of the Society will be published in the November issue. Such abstracts must not exceed 250 words in length, should be typed (double spaced) and the ribbon copy submitted, the lines on the page should be numbered, in the left margin, from top to bottom, and must be in the hands of the Secretary of the Society by August 15, preceding the meeting. These abstracts must be summaries of results and conclusions.

10. Presentation of a paper at the annual meeting does not exclude it from publication in complete form in the JOURNAL. Such papers may be submitted to the Editor and will be given equal consideration with others submitted.

11. All manuscripts and correspondence concerning them should be addressed to the Editor: Dr. W. D. Gallup, Department of Biochemistry, Oklahoma State University, Stillwater, Oklahoma, or to the publishers: Boyd Printing Company, 372–378 Broadway, Albany 7, New York.

12. All correspondence concerning subscriptions to the JOURNAL OF ANIMAL SCIENCE and other business matters should be addressed to the Business Manager, Dr. H. H. Stonaker, Department of Animal Husbandry, Colorado State University, Fort Collins, Colo., or to the publishers, Boyd Printing Company, 372–378 Broadway, Albany 7, New York.

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